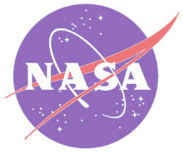
International Technology Research Institute
World Technology (WTEC) Division


WTEC Panel Report on

TISSUE ENGINEERING RESEARCH

Larry V. McIntire (Panel chair)
Howard P. Greisler
Linda Griffith
Peter C. Johnson
David J. Mooney
Milan Mrksich
Nancy L. Parenteau
David Smith

January 2002

International Technology Research Institute
R.D. Shelton, Director
Geoffrey M. Holdridge, WTEC Division Director and Series Editor

4501 North Charles Street
Baltimore, Maryland 21210-2699

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JAN 2002		**00-00-2002 to 00-00-2002**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Tissue Engineering Research	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **International Technology Research Institute,4501 North Charles Street,Baltimore,MD,21210-2699**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**		**232**	

WTEC PANEL ON TISSUE ENGINEERING RESEARCH

Sponsored by the National Science Foundation, Defense Advanced Research Projects Agency, National Aeronautics and Space Administration, National Institute of Standards and Technology, National Institutes of Health, and the Food and Drug Administration of the United States Government.

Larry V. McIntire, Ph.D.
E.D. Butcher Professor and Chair
Institute of Biosciences and Bioengineering
Rice University
MS 144, 6100 Main Street
Houston, TX 77005-1892

Howard P. Greisler, M.D.
Department of Surgery
Department of Cell Biology, Neurobiology, and Anatomy
Loyola University Medical Center
2160 South First Avenue
Maywood, IL 60153
and
Hines VA Hospital
Office of Research and Development
5th Ave. and Roosevelt Road
Hines, IL 60141

Linda Griffith, Ph.D.
Chemical Engineering Department and Division of Bioengineering and Environmental Health
MIT
Cambridge, MA 02139-4307

Peter C. Johnson, M.D.
President and Chief Executive Officer
TissueInformatics, Inc.
711 Bingham St., Suite 202
Pittsburgh, PA 15203

David J. Mooney, Ph.D.
Department of Chemical Engineering,
Department of Biologic & Materials Sciences
University of Michigan
2300 Hayward St.
Room 3074 H.H. Dow Bldg.
Ann Arbor, MI 48109-2136

Milan Mrksich, Ph.D.
Dept of Chemistry
SCL-463
University of Chicago
5801 S. Ellis Ave.
Chicago, IL 60637

Nancy L. Parenteau, Ph.D.
Senior Vice President and Chief Scientific Officer
Organogenesis, Inc.
150 Dan Road
Canton, MA 02021

David Smith, J.D.
General Counsel
TissueInformatics, Inc.
711 Bingham St., Suite 202
Pittsburgh, PA 15203

Special thanks to the following additional members of the traveling party (including sponsor representatives) who contributed site reports to this volume: Frederick Heineken (NSF), Christine Kelley (NIH), Alan Russell (University of Pittsburgh), William Wagner (University of Pittsburgh), and Jennifer West (Rice University).

INTERNATIONAL TECHNOLOGY RESEARCH INSTITUTE

World Technology (WTEC) Division

WTEC at Loyola College (previously known as the Japanese Technology Evaluation Center, JTEC) provides assessments of foreign research and development in selected technologies under a cooperative agreement with the National Science Foundation (NSF). Loyola's International Technology Research Institute (ITRI), R.D. Shelton, Director, is the umbrella organization for WTEC. Elbert Marsh, Deputy Assistant Director for Engineering at NSF's Engineering Directorate, is NSF Program Director for WTEC. Several other U.S. government agencies provide support for the program through NSF.

WTEC's mission is to inform U.S. scientists, engineers, and policymakers of global trends in science and technology in a manner that is timely, credible, relevant, efficient and useful. WTEC assessments cover basic research, advanced development, and applications. Panels of typically six technical experts conduct WTEC assessments. Panelists are leading authorities in their field, technically active, and knowledgeable about U.S. and foreign research programs. As part of the assessment process, panels visit and carry out extensive discussions with foreign scientists and engineers in their labs.

The ITRI staff at Loyola College help select topics, recruit expert panelists, arrange study visits to foreign laboratories, organize workshop presentations, and finally, edit and disseminate the final reports.

WTEC has now been spun off to a private, nonprofit corporation that will conduct all future WTEC studies, while continuing to assist in dissemination of older WTEC reports. See http://www.wtec.org.

Dr. R.D. Shelton
ITRI Director
Loyola College
Baltimore, MD 21210

Mr. Geoff Holdridge
WTEC Division Director
Loyola College
Baltimore, MD 21210

Dr. George Gamota
ITRI Associate Director
17 Solomon Pierce Road
Lexington, MA 02173

National Science and Technology Council
Multi-Agency Tissue Engineering Science (MATES) Working Group

TISSUE ENGINEERING RESEARCH

A World Technology Evaluation Center (WTEC) Panel Report

Dear Colleague:

As America enters the 21st century and a new age, strategic investments in science and engineering will be increasingly important determinants in enabling us to meet threats to our national security, improve the health and quality of life for our citizens, and maintain our economic strength and our overall leadership in the civilized world. The next 5 to 10 years will be critical for the maturation of tissue engineering and its pivotal role in clinical medicine. Tissue engineering, a multidisciplinary science that emerged from discovery research in the 1970s, has evolved towards applications for the repair and regeneration of diseased or damaged tissues. The 1990s witnessed the development of products for a variety of different medical conditions, affecting virtually every organ system in the body; some have been approved for clinical use, while many are still under investigation or evaluation. This study by the World Technology Evaluation Center (WTEC) provides a basis for developing future national research and development (R&D) priorities and formulating a strategy for effective Federal Government support in the field of tissue engineering. The purpose of this study was to gather information on tissue engineering research in Japan and Europe compared to that in the United States and to assist the Multi-Agency Tissue Engineering Science (MATES) Working Group of the National Science and Technology Council in determining if the Federal Government is providing the appropriate strategic R&D investments in this emerging field. The findings of the WTEC study will assist MATES member agencies in guiding the Federal tissue engineering research agenda, assuring the continued maturation of the field to its full potential.

The final report from this effort, *Tissue Engineering Research - A WTEC Panel Report*, highlights new developments in biomaterials, bioinformatics, imaging and related areas of computer science, cell biology research, as well as non-medical applications such as novel methods for detection and remediation of biological and chemical threats. In its comparative review of research programs in the United States, Europe, and Japan, the report provides a broad perspective on research directions in tissue engineering worldwide. While the United States maintains its lead in the field, major new government-funded research programs in both Europe and Japan are challenging the U.S. lead.

This document will serve as a basis for continued dialogue within our nation's tissue engineering R&D community and with other important stakeholders, providing guidance for future programs. It highlights the necessity for providing continued and enhanced resources to further the progress in tissue engineering, harness new developments, and maintain our scientific and economic leadership.

Sincerely yours,

Kiki B. Hellman, Ph.D.
Co-Chair
MATES Working Group

Fred G. Heineken, Ph.D.
Co-Chair
MATES Working Group

WTEC Panel on

TISSUE ENGINEERING RESEARCH

FINAL REPORT

January 2002

Larry V. McIntire (panel chair)
Howard Greisler
Linda Griffith
Peter C. Johnson
David J. Mooney
Milan Mrksich
Nancy L. Parenteau
David Smith

This document was sponsored by the National Science Foundation (NSF), the Defense Advanced Research Projects Agency, the National Institute of Standards and Technology, the National Aeronautics and Space Administration, the National Institutes of Health, and the Food and Drug Administration of the U.S. Government under NSF Cooperative Agreement ENG-9707092 and NSF Grant ENG-0104476. The government has certain rights in this material. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the United States Government, the authors' parent institutions, or Loyola College.

ABSTRACT

This report is a comparative review of tissue engineering research and development activities in the United States, Japan, and Western Europe conducted by a panel of leading U.S. experts in the field. It covers biomaterials, cells, biomolecules, non-medical applications, engineering design, informatics, and legal and regulatory issues associated with tissue engineering research and applications. The panel's conclusions are based on a literature review, a U.S. review workshop held at NIH in June of 2000, and a series of site visits to leading tissue engineering research centers in Japan and Western Europe. A summary of the June 2000 workshop is included as an appendix, as are site reports from each of the panel's overseas visits. An executive summary is included conveying the panel's overall conclusions.

ACKNOWLEDGEMENTS

I would like to thank the U.S. Government sponsors of this study: Frederick Heineken, Bruce Hamilton, Lynn Preston, and Elbert Marsh of NSF; Christine Kelley, John Watson, and Dick Swaja of NIH; Alan Rudolph, Kurt Henry, and Joseph Bielitzki of DARPA; Rosemarie Hunziker, Hoda Elgendy, and Angela Hight-Walker of NIST; Stephen Davison of NASA; and Kiki Hellman, Darin Weber, and Joyce Frey-Vasconcells of FDA. Special thanks are due to Rosemarie Hunziker of NIST, who took the lead in preparing Appendix E to this report, summarizing U.S. government funding for tissue engineering research and development, in cooperation with staff from the sponsoring agencies and the members of the Multi-Agency Tissue Engineering Science (MATES) Working Group. We are very much indebted to our panel chair, Larry McIntire of Rice University and to all the members of the panel for their invaluable contributions of time and intellect to this study. It was indeed an honor to work with such a wonderful group. Special thanks are due to Alan Russell of the Pittsburgh Tissue Engineering Initiative (and of the University of Pittsburgh, McGowan Institute for Regenerative Medicine), William Wagner of the University of Pittsburgh, and Jennifer West of Rice University for traveling with the panel and contributing site reports to this volume and their insights to the study in general. Finally, we are extremely grateful to all of our hosts and correspondents around the world who took the time to share their work with us, as well as their vision of the future of this exciting field.

Sincerely,

Geoffrey M. Holdridge
Vice President for Operations, WTEC, Inc., and ITRI Series Editor

International Technology Research Institute (ITRI)

R. D. Shelton, Principal Investigator, ITRI Director

World Technology (WTEC) Division

Geoffrey M. Holdridge, WTEC Division Director and ITRI Series Editor
Bobby A. Williams, Financial Officer
Roan E. Horning, Web Administrator
Robert Tamburello, Professional Assistant

Aminah Grefer, Global Support Inc., Europe Advance Contractor
Gerald Whitman, ENSTEC, Inc., Japan Advance Contractor
Stephen B. Gould, ITRI, Inc., Staff Representative, Europe Trip
Hiroshi Morishita, WTEC Japan Representative
Patricia M. Johnson, Editor

FOREWORD

> We have come to know that our ability to survive and grow as a nation to a very large degree depends upon our scientific progress. Moreover, it is not enough simply to keep abreast of the rest of the world in scientific matters. We must maintain our leadership.[1]

President Harry Truman spoke those words in 1950, in the aftermath of World War II and in the midst of the Cold War. Indeed, the scientific and engineering leadership of the United States and its allies in the twentieth century played key roles in the successful outcomes of both World War II and the Cold War, sparing the world the twin horrors of fascism and totalitarian communism, and fueling the economic prosperity that followed. Today, as the United States and its allies once again find themselves at war, President Truman's words ring as true as they did a half century ago. The goal set out in the Truman Administration of maintaining leadership in science has remained the policy of the U.S. Government to this day: Dr. John Marburger, the Director of the Office of Science and Technology (OSTP) in the Executive Office of the President made remarks to that effect during his confirmation hearings in October 2001.[2] The OSTP Web site states that "the Federal Government plays a critical role in maintaining American leadership in science and technology."[3]

The United States needs metrics for measuring its success in meeting this goal of maintaining leadership in science and technology. That is one of the reasons that the National Science Foundation (NSF) and many other agencies of the U.S. Government have supported the World Technology Evaluation Center (WTEC) and its predecessor programs for the past 19 years. While other programs have attempted to measure the international competitiveness of U.S. research by comparing funding amounts, publication statistics, or patent activity, WTEC has been the most significant public domain effort in the U.S. Government to use peer review to evaluate the status of U.S. efforts in comparison to those abroad. Since 1983, WTEC has conducted over 50 such assessments in a wide variety of fields, from advanced computing, to nanoscience and technology, to biotechnology.

The results have been extremely useful to NSF and other agencies in evaluating ongoing research programs, and in setting objectives for the future. WTEC studies also have been important in establishing new lines of communication and identifying opportunities for cooperation between U.S. researchers and their colleagues abroad, thus helping to accelerate the progress of science and technology generally within the international community of civilized nations. Just as many of the scientific and technological triumphs of the World War II and Cold War eras were accomplished through international cooperation between the United States and its allies, so our continued progress in science and technology depends on unfettered communication and cooperation among friendly nations. Finally, WTEC is an excellent example of cooperation and coordination among the many agencies of the U.S. Government that are involved in funding research and development: almost every WTEC study has been supported by a coalition of agencies with interests related to the particular subject at hand. In some cases, these coalitions formed to support a WTEC study have outlived the studies themselves, evolving into ongoing cooperative efforts among the agencies involved.

The present study, reviewing the status of tissue engineering research and development in the United States, Japan, and Europe, is a case in point. Support for this study came from NSF, agencies of the Department of Health and Human Services (the National Institutes of Health and the Food and Drug Administration), the National Institute of Standards and Technology, the Defense Advanced Research Projects Agency, and the National Aeronautics and Space Administration. It has been a focal point over the past 18 months for the activities of the Multi-Agency Tissue Engineering Science (MATES) interagency working group, under the

---

[1] Remarks by the President on May 10, 1950 on the occasion of the signing of the law that created the National Science Foundation. *Public Papers of the Presidents* 120: p. 338.

[2] http://www.ostp.gov/html/01_1012.html.

[3] http://www.ostp.gov/html/OSTP_insideostp.html.

auspices of the Subcommittee on Biotechnology, Committee on Science of the President's National Science and Technology Council (NSTC). The results of the WTEC study are being used now by MATES to plan a joint interagency program announcement in tissue engineering. MATES represents the first effort to coordinate tissue engineering research and development activities within the Federal Government. Formally established in January of 2000, it is charged with facilitating communication across departments/agencies by regular information exchanges and a common web site (http://tissueengineering.gov), enhancing cooperation through co-sponsorship of scientific meetings and workshops, facilitating the development of standards, and monitoring technology by undertaking cooperative assessments of the status of the field. As recognized by a recent National Academy of Sciences report[4], international benchmarking studies can be an important tool for strategic planning by U.S. Government agencies. The MATES group therefore embraced the WTEC study as a key element in carrying out its mission.

It would be difficult to overstate the promise of this exciting new field of tissue engineering. Starting from a few modest NSF grants in the mid-1980s, followed by major funding from NIH and NIST, the field has spawned a burgeoning industry that has enjoyed over $3 billion in funding over the past decade, much of it from private sources.[5] According to the WTEC panel, the United States maintains a lead in tissue engineering, particularly in privately funded applied research; however, governments in both regions have initiated major new research programs in this area that will challenge the U.S. lead. The panel also found that Japan offers new insights in biomaterials, and Europe is providing strong support for basic cell biology research that is the underpinning for future progress in the field. In the long term, tissue engineering offers the promise of revolutionizing health care, prolonging and improving the quality of life for millions of people around the world, and greatly reducing the cost of treating debilitating diseases such as diabetes, heart disease, and liver failure. In the near term, tissue engineering is already having an important impact in treatment of skin ulcers and burns. Perhaps most notable in the context of our current international crisis, tissue engineering is being used today to develop new ways of detecting biological threats (as documented in Chapter 5 of this report), and may offer promise in the future of helping remediate such threats. Even the very first FDA-approved tissue engineered medical products have had an impact on our ability to respond to the threat of global terrorism: living engineered tissue (Apligraf®[6]) was donated by Organogenesis, Inc. to treat burn victims from the World Trade Center attack.[7]

As we seek to refine the WTEC activity, improving the methodology and enhancing the impact, the program will continue to operate from the same basic premise that it has from its inception: improved awareness of international developments in science and technology can help inform U.S. research funding decisions, and can significantly enhance the scope and effectiveness of international scientific collaboration. This in turn contributes to the security, health, and economic well being of the United States and the entire world. As President Truman said over 50 years ago, our very survival depends upon continued leadership in science and technology. WTEC plays a key role in determining whether the United States is meeting that challenge.

Elbert Marsh
Deputy Assistant Director for Engineering
National Science Foundation

---

[4] Chemical and Engineering News, March 20, 2000.

[5] Michael Lysaght in *Proceedings of the WTEC Workshop on Tissue Engineering Research in the United States*: http://www.wtec.org/loyola/te/usws/usws-00.pdf.

[6] Apligraf is a registered trademark of Novartis Pharmaceuticals.

[7] *The Patriot Ledger*, September 15, 2001.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

Larry V. McIntire

INTRODUCTION

Tissue engineering is defined as the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and pathological mammalian tissues, and the development of biological substitutes to restore, maintain, or improve tissue function. Sometimes also called reparative and regenerative medicine, tissue engineering is an emerging interdisciplinary area of research and technology development that has the potential to revolutionize methods of health care treatment and dramatically improve the quality of life for millions of people throughout the world. Some products are already in use clinically, and their number will assuredly increase rapidly in the future.

A worldwide study of the status and trends in tissue engineering research and development was carried out during the period 1999-2001 by an eight-person panel under the auspices of the World Technology (WTEC) Division of the International Technology Research Institute at Loyola College in Maryland. Led by the National Science Foundation, a wide range of U.S. Government organizations commissioned this study: the National Institutes of Health, the National Institute for Standards and Technology, the Defense Advanced Research Projects Agency, the National Aeronautics and Space Administration, and the Food and Drug Administration. This support indicates the breadth of interest in and immense potential of this rapidly growing new field. The purpose of this study was to gather and disseminate information for the U.S. tissue engineering and science policy communities on the current status and future trends in research and development in the field of tissue engineering in Europe and Japan, in comparison to U.S. activity in this field. The goals included the following:

1. Gain a broader understanding of the work being performed globally in the design, fabrication, and use of engineered tissues by identifying, visiting, and assessing the work at key research centers
2. Reveal new cross-disciplinary strategies that are being used to advance novel research approaches to specific application areas within the field of tissue engineering, including exploration of models of cooperation across industry, government, and academia in different countries
3. Examine the scientific basis for advancing methodologies focused on evaluating the cellular response to implants, the quality and fabrication of implants, and human acceptance
4. Assess the effect of the regulatory environment on progress of critical work in tissue engineering
5. Identify and encourage opportunities for international collaboration in this emerging field

This executive summary of the WTEC panel's final report presents an overview of the panel's observations and conclusions regarding tissue engineering science and technology worldwide. The chapters written by panel members report on critical areas that form the building blocks necessary for substantial growth of the tissue-engineering field. Site reports documenting the panel's visits to university, government, and industry laboratories in Europe and Japan are included in this volume as appendices. A companion report, also available from WTEC, contains the proceedings of a WTEC tissue-engineering workshop held on the NIH campus in Bethesda, Maryland, on June 5-6, 2000, with the purpose of assessing the current state of the U.S. tissue-engineering enterprise.

FINDINGS

Table ES.1 summarizes the panel's comparisons between U.S., Japanese, and European tissue-engineering R&D activities, at a fairly gross level of generalization. In additional, several other general conclusions can be drawn from the information assembled in the WTEC Study:

1. Until recently, most of the funding to support activities in tissue engineering in the United States has been in support of commercial development (companies, NIST/ATP Program), leading to large amounts of applied research, but lesser amounts of fundamental research. In Japan and Europe, the tissue engineering field is being largely driven by government funding, allowing researchers to perform more basic research, which offers greater potential for generating intellectual property. Examples include London's Imperial College Tissue Engineering Center with its focus on stem cell research and the new Manchester/Liverpool Tissue Engineering Center, built on the foundations of the long standing Welcome Trust-funded Centre for Cell-Matrix Research.

2. Use of autologous cells is predominant in both Europe and Japan. In Europe there was surprisingly little discussion of the development of allogeneic cell products. Allogeneic products are amendable to large-scale manufacturing at single sites, while autologous therapies will likely lead to more of a service industry, with a heavy emphasis on local or regional cell banking/expansion. In the United States, both autologous and allogeneic cell products are being developed, but the largest companies (e.g., Advanced Tissue Sciences and Organogenesis) are focused on allogeneic products. Different technologies will be needed to achieve success in these two different models.

3. Many centers of tissue engineering in both Europe and Japan devote much of their efforts to challenges in cell technologies, often combining cells with existing materials in clinically driven application approaches to regenerating tissues. Many of these tissue engineering programs employ off-the-shelf biomaterials, with the aim of creating novelty through applications of cells, and thus do not explicitly focus on development of new biomaterials or even on significant modification of existing biomaterials. In general, the United States leads in the development of novel biomaterials. There are several important exceptions in both Europe and Japan, however, where there is a focus on utilization of biological molecular design principles, including a fairly sophisticated knowledge of receptor-mediated cell interactions, to develop new and novel biomaterials.

4. Rapid advances in the tissue-engineering field will require linkage between basic biological scientists, bioengineers and material scientists, and clinical researchers. The United States is currently ahead in generating these cross-disciplinary environments, but there is strong movement in both Europe and Japan to promote the interactions among different laboratories specifically to advance tissue-engineering applications, often by establishing centers with links to private industry. Examples include the tissue-engineering aspects of the Japanese Millenium Project and the UK Manchester/Liverpool Tissue Engineering Centre.

5. The United States has a leadership position in the genomics-based development of databases to which data mining tools can be directed for drug discovery. The use of informatics approaches in tissue engineering is in its infancy. Notable exceptions to the absence of informatics solutions for tissue engineering approaches were found at Keio University, where the e-Cell initiative is pursuing goals similar to those being undertaken by many U.S. universities and by Physiome Sciences—that is, the development of a computer model of a virtual cell. At Heidelberg, the European Molecular Biology Laboratory has long been a leader in the application of informatics solutions to biological problems, particularly in the area of molecular analysis and genomics. With its development of the BioImage database, an increasing interest has been shown in the role of shared tissue images and related information in the understanding of the mechanism of disease; however, the direct application to tissue engineering has apparently not been organized. Several institutions have ongoing functional genomics activities and 3D modeling activities, but in most cases these remain confined to the genomics sector.

Table ES.1.
Comparisons Among U.S., Japanese, and European Tissue Engineering R&D Efforts

	Topic	Knowledge Base	Work to Date	Leading Region
Biomaterials	Adapted biomaterials	Advanced	Extensive	Equivalent
	Adapted bioactive materials	Advanced	Extensive	Equivalent
	Biomaterial design	Incomplete	Extensive	U.S.>Europe>Japan
	Linkage of biomaterial Design to cell biology/ development	Incomplete	Modest	U.S.
	Clinical application of novel concepts	Incomplete	Little	Equivalent
Cells	Enabling methodology	Moderate	Moderate	Equivalent
	Allogeneic cells/ immunological manipulation	Extensive	Active in U.S. Modest in EU Little in Japan	U.S.
	Stem cell research	Extensive in hematopoietic system	Widely dispersed activity	Equivalent
	Commercialization of cell therapies	Moderate	Extensive activity in U.S. Modest in EU Early in Japan	U.S.
Biomolecules	Gene transfer	Incomplete	Extensive	U.S.
	Angiogenic factors	Incomplete	Limited	I.D. of factors: U.S. Delivery of factors: equivalent
	Growth factors	Extensive	Moderate	I.D. of factors: U.S. Delivery of factors: equivalent
	Differentiation factors	Little	Limited	Too early to determine
	BMPS	Incomplete	Moderate	U.S. (close)
Engineering Design (Mass Transport)	2-d cell expansion	Advanced	Extensive	U.S.
	3-d tissue growth	Incomplete	Significant	U.S.
	Liver devices	Little	Significant	Equivalent
	Promoting vascularization	Incomplete	Little	Too early to determine
	Cell storage	Advanced	Extensive	Equivalent
	Tissue storage	Incomplete	Little	Too early to determine
Engineering Design (Biomechanics)	Properties of native tissues	Incomplete	Extensive	Equivalent
	ID minimum props. of engineered tissues	Little	Little	Too early to determine
	Biomechanics input to cells	Advanced	Significant	Equivalent
	Biomechanics input to eng. tissues	Incomplete	Little	U.S.
Informatics	Genomics	Advanced	Extensive	U.S.>UK>Switz
	Proteomics	Incomplete	Significant	U.S.
	Microarray	Advanced	Extensive	U.S.
	Cell informatics	Incomplete	Significant	U.S.
	Tissue informatics	Little	Little	U.S., Germany
	Physiome (system)	Incomplete	Significant	U.S.>Japan
	Commercial	Incomplete	Significant	U.S.>Germany
Cell-Based Tech., Non-Medical Apps.	Cell-based sensors	Moderate	Significant	U.S.
	Neural networks	Incomplete	Significant	Equivalent
	Other applications	Incomplete	Little	Too early to determine
	Engineering active interfaces	Incomplete	Little	Equivalent

6. A number of engineering areas/technologies will be critical to developing tissue-engineered products. These include bioreactor design, optimization of mass transport following cell transplantation, understanding of the biomechanical requirements of engineered tissues, and using electrical/mechanical stimulation to promote desired development of engineered tissues. A great deal of work has been done in the United States to develop novel bioreactors for expansion of a variety of cell types and sources, both in 2D and 3D culture. The potential importance of autologous cell therapies in Europe and Japan will demand significant attention to this topic, but most bioreactor work in these regions currently follows the U.S. lead. The importance of vascularization to enhance mass transport in engineered tissues is widely acknowledged, but little progress has been made to date in any region. The biomechanics issues in tissue engineering have not been addressed to the extent that biochemistry issues (e.g., composition of tissues, protein secretion) have been in the past. Little is known regarding the necessary or desired mechanical properties of many potential tissue-engineering products. Compared to Europe or Japan, the potential role of mechanical signals in tissue development has been explored in the United States to a greater, albeit still very limited, extent.

7. Regulatory issues present a major challenge to the worldwide development of the tissue engineering industry. The FDA approach to the regulation of products incorporating human tissues is comprehensive but not fully implemented. In the absence of a European Union regulatory program, those European governments that have addressed the status of engineered tissue products have employed an array of classification schemes that further complicate international application of this technology. Like a number of European states, Japan has yet to articulate its own regulatory policy. Uncertainty in classification between states and, with that, unpredictability in marketing approval strategies may impede product development, especially in the case of engineered tissues developed for smaller patient populations.

The implications of governmental authority over access to human tissues for research and development purposes are equally clouded by multiple responses to the legal, ethical, and cultural issues, with the recent debate over the use of embryonic stem cells highlighting these different approaches. In both Europe and Japan, the availability of tissues within academic institutions and their researchers' ability to employ manipulated tissues in small-scale applications in humans contrasted with the barriers faced by commercial entities in acquiring tissues (especially in Japan) and the greater scrutiny given to their clinical uses of engineered tissues. Differentiating between academic and industrial uses of research tissues may ameliorate possible concerns over the commercialization of tissue transfer, although it may slow the scaling of new tissue-engineering technologies to meet regulatory approval requirements.

In order for the immense potential of tissue engineering to be realized in the United States, an intensive national effort will be required to provide the basic structure-function relationships from the molecular to the tissue level and to develop the engineering systems and analysis needed to produce functional tissue replacements. Developing focused large-scale initiatives to fill the gap areas in basic science and engineering will be crucial for the United States if it is to continue to lead in the development of actual products for this exceptionally important emerging field. As our population ages, tissue engineering and regenerative medicine will become important economic forces, and the United States must be prepared to lead.

CHAPTER 1

INTRODUCTION

Larry V. McIntire

BACKGROUND: THE SIGNIFICANCE OF TISSUE ENGINEERING

Tissue engineering as defined in this report is "the application of principles and methods of engineering and life sciences to obtain a fundamental understanding of structure-function relationships in novel and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve [tissue] function" (Skalak and Fox 1988). This is an emerging interdisciplinary area of research and technology development that has the potential to revolutionize our methods of health care treatment and dramatically improve the quality of life for millions of people throughout the world. As an indication of the scope of the problem that tissue engineering addresses, worldwide organ replacement therapies utilizing standard organometallic devices consume 8 percent of medical spending, or approximately $350 billion per year (Lysaght and O'Loughlin 2000). Organ transplantation is another option, but one that is severely limited by donor availability. Tissue-engineered products hold the promise of true functional replacement at affordable cost.

HISTORY OF TISSUE ENGINEERING

The early practice of medicine relied largely on palliative management of pain and distress. As science contributed to this art, pharmaceuticals to change the body's physiology, vaccines to prevent communicable diseases, or surgery to remove diseased parts became, and largely remain, the standard medical therapies. Until very recently, most scientists and clinicians believed that damaged or diseased human tissue could only be replaced by donor transplants or with totally artificial parts. Tissue engineering promises a more advanced approach in which organs or tissues can be repaired, replaced, or regenerated for more targeted solutions. This approach also responds to clinical needs that cannot be met by organ donation alone.

The term "tissue engineering" was coined at a meeting sponsored by the National Science Foundation (NSF) in 1987. At a subsequent NSF-sponsored workshop, tissue engineering was formally defined as noted in the first sentence of this chapter. Other definitions exist. Langer and Vacanti (1993) defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function." Galletti, Hellman, and Nerem (1995) defined tissue engineering as "the basic science and development of biological substitutes for implantation into the body or the fostering of tissue remodeling for the purpose of replacing, repairing, regenerating, reconstructing, or enhancing function." These subsequent definitions essentially reiterate the NSF definition. Two other recently popular terms, *regenerative medicine* and *reparative biology*, have considerable, sometimes total, overlap with the aims and goals of tissue engineering.

PROMISE OF TISSUE ENGINEERING

Tissue engineering as a multidisciplinary science to restore biological function, either through repair or regeneration, has led to a broad range of potential products based on their common source materials:

- human tissues or organs (e.g., autologous or allogeneic tissues)
- animal tissues or organs (e.g., transgenic animals or xenotransplants)
- processed, selected, or expanded human or other mammalian cells (e.g., stem/progenitor cells, genetic, and somatic cellular therapies), with or without biomaterials
- totally synthetic materials of biomimetic design

Representative products of these source material classes are in different stages of development and include both structural/mechanical substitutes and metabolic substitutes. Structural/mechanical substitutes include artificial skin constructs; expanded cells for cartilage regeneration; engineered ligament and tendon; bone graft substitutes/bone repair systems; products for nerve regeneration; engineered cornea and lens; and products for periodontal tissue repair. Metabolic substitutes include implanted, encapsulated pancreatic islet cells; *ex vivo* systems such as extracorporeal liver assist devices; engineered products for cardiovascular repair/regeneration; blood substitutes; and encapsulated cells for restoration of tissue/organ function, other than encapsulated islet cells used as implants or encapsulated hepatocytes used as *ex vivo* metabolic support systems (Hellman, Knight, and Durfor 1998; Hellman et al. 2000; Bonasser and Vacanti 1998). To date, a few of these products have been approved by the U.S. Food and Drug Administration (FDA) while many are under either preclinical investigation or regulatory evaluation (Hellman, Knight, and Durfor 1998; Hellman et al. 2000).

OTHER APPLICATIONS

Cell-based tissue-engineered systems may also be utilized as exceptionally sensitive "sensors." Applications could include detection of infrared signals from great distances and development of predictive models for toxicity assessment. The combination of cells and silicone based technology also holds great promise for development of *in vitro* neural networks and novel computational device development. As research tools, these systems could also be employed as correlates of *in vitro* and *in vivo* biological activity.

EMERGING INDUSTRY OF TISSUE ENGINEERING

In a little over a decade, more than $3.5 billion has been invested in worldwide research and development in tissue engineering. Over 90% of this financial investment has been from the private sector (Lysaght and Reyes 2001). Currently there are over 70 start-up companies or business units in the world, with a combined annual expenditure of over $600 million dollars. Tissue-engineering firms have increased spending at a compound annual rate of 16% since 1990. An interesting recent tend has been the emergence of significant activity in tissue engineering outside the United States. At least 15 European companies are now active (Lysaght, MJ, and Reyes 2001). Impressive government investment in tissue engineering (“regenerative medicine”) has been made recently by Japan (through its Millennium Project) and by several European initiatives.

OBJECTIVES OF THE WTEC STUDY

Given the rapid development of tissue-engineering research in the United States and abroad, several U.S. government agencies, under the leadership of the National Science Foundation (NSF), asked the World Technology Evaluation Center (WTEC) to conduct a worldwide comparative survey of tissue engineering R&D. The stated purpose of the study was to document R&D activities in the United States and abroad aimed at developing a better understanding of the design, fabrication, and use of engineered tissues to replace parts of a living system or to function extracorporally. The broad objectives of the study were defined by the sponsors as follows:

- Gain a broader understanding of the work being performed globally in the design, fabrication, and use of engineered tissues by identifying, visiting, and assessing the work at key research centers
- Explore more closely the highly innovative technological advances and breakthroughs rather than incremental improvements
- Examine the scientific basis for advancing methodologies focused on evaluating the cellular responses to implants, the quality and lifetime of implants, and assessing human acceptance
- Reveal new cross-disciplinary strategies and funding mechanisms that are being utilized to advance novel research approaches to specific application areas within the field of tissue engineering
- Identify opportunities for international collaboration in this emerging field
- Assess the effect of the regulatory environment on the progress of critical work

The sponsors directed WTEC to recruit a panel of U.S.-based experts in various aspects of tissue engineering to carry out this study. Details of the study scope and methodology were left open to discussion among WTEC staff, sponsors, and panelists.

SCOPE AND METHODOLOGY

Panel Recruitment

After extensive discussions with sponsors at the National Science Foundation, the Defense Advanced Research Projects Agency (DARPA), the National Institutes of Health (NIH), the National Institute of Standards and Technology (NIST), the National Aeronautics and Space Administration (NASA), and the Food and Drug Administration (FDA), WTEC asked this author to chair the panel and lead the study effort. After meeting with sponsors to discuss their requirements, we then recruited the following panel of experts to carry out the study:

- Linda Griffith, MIT
- Howard Greisler, Loyola University Medical Center
- Peter Johnson, TissueInformatics, Inc.
- David Mooney, University of Michigan
- Milan Mrksich, University of Chicago
- Nancy Parenteau, Organogenesis, Inc.
- David Smith, Reed Smith Shaw & McClay, LLP (at the time; now at TissueInformatics)

Robert Langer of MIT also agreed to serve as an advisor to the panel, as did Jeffrey Hubbell of ETH/Zurich. Biographies of the panel members are included in Appendix A of this report.

Study Scope

The detailed definition of the scope of the study was determined at a kickoff meeting held at the National Science Foundation in February 2000. Following extensive discussion among sponsors, staff, and panelists, the following report outline was agreed upon:

1. Executive Summary and Introduction (Larry McIntire)
2. Biomaterials (Linda Griffith)
3. Cells (Nancy Parenteau)
4. Biomolecules (Howard Greisler)
5. Engineering (David Mooney)
6. Cell Based Sensors and other Non-Medical Applications (Milan Mrksich)
7. Informatics (Peter Johnson)
8. Legal and Regulatory Issues (David Smith)

Methodology

Also at the February 2000 kickoff meeting, study participants discussed details of how the study would be carried out. The group agreed on the following approach:

1. To provide a baseline of information on U.S. activities as a starting point for the study, WTEC organized a workshop at NIH on June 5-6, 2000, at which key U.S. researchers in each of the above topical areas were invited to make presentations on their current activities and summarize other U.S. activities in their areas. WTEC immediately produced a draft proceedings report from the workshop for distribution to sponsors and overseas researchers whom the panel visited later in the summer. Following the workshop, participants were invited to revise and extend their remarks in written form. The final report from that that workshop is available on the Internet at http://itri.loyola.edu/te/usws/welcome.htm.

2. The panel visited key researchers and government agencies in Europe during the week of July 15-23, and in Japan during the week of August 18-27, 2000. In all, the panel visited 41 sites in Japan and Europe; some of these meetings included researchers from additional sites that the panel did not have time to visit in person. In addition to the panel members, the traveling study teams for these trips included WTEC staff representatives and sponsor representatives. Because Linda Griffith and Peter Johnson were unable to make all the overseas trips, Jennifer West of Rice University, Alan Russell of the University of Pittsburgh, and William Wagner of the University of Pittsburgh also participated in the site visits. Site reports detailing what the panel learned during these visits are included in Appendix B of this report. In addition to panel members, Jennifer West, Alan Russell, William Wagner, Christine Kelley (NIH) and Frederick Heineken (NSF) contributed site reports to this volume. WTEC sent each site visit host a draft of his or her site report for review and correction prior to its inclusion in this draft report.

3. The panel presented preliminary findings from the study at a workshop held at NIST on November 2-3, 2000. Participants in the June 2000 U.S. review workshop were invited, as were overseas site visit hosts, the tissue-engineering research community, and the general public. The first day of the workshop included technical presentations on each of the above outline topics by the members of the panel. Participants were invited to ask questions and comment on the presentations. The second day of the workshop featured brief presentations from senior representatives of the sponsoring agencies, reacting to the panel's findings and discussing possible U.S. government responses. Viewgraphs from the panelists' presentations at that workshop are available at http://itri.loyola.edu/te/views/top.htm.

4. Following the workshop, panel members each prepared written analytical chapters reviewing the status of U.S. and overseas activities in their respective subtopics of the study. These chapters comprise the body of this report. They cover the material reviewed in the November workshop proceedings, but in more detail. Preparing these chapters after the November workshop allowed panelists to incorporate into the report comments they received from workshop participants.

5. The full draft report was sent for review to all U.S. and foreign participants in the study. They were invited to suggest improvements in the report and to correct any factual statements concerning their respective activities and organizations.

6. Following review by sponsoring agencies, U.S. workshop participants, overseas site visit hosts, and a technical editor, the final report is published by WTEC both in print and on the Web. All study participants receive printed copies; the full report is available for free download on the Web at http://www.wtec.org/loyola/te/ or http://itri.loyola.edu/te/.

VISION FOR THE FUTURE

The following chapters give detailed evaluations of the current status of various aspects of tissue engineering progress in Europe, Japan, and the United States. Each chapter also identifies areas where there are gaps in our basic science and engineering knowledge base that inhibit rapid progress towards functional products in tissue engineering or regenerative medicine.

In order for the immense potential of tissue engineering to be realized in the United States, an intensive national effort will be required to provide the basic structure-function relationships from the molecular to the tissue level and to develop the engineering systems and analysis needed to produce functional tissue replacements. Several centers of excellence in tissue engineering have evolved: Harvard-MIT, Georgia Tech-Emory University, Rice University-Texas Medical Center, and the Universities of Pittsburgh and Michigan, for example. Developing focused large-scale initiatives to fill the gap areas in basic science and engineering will be crucial for the United States if it is to continue to lead in the development of actual products for this exceptionally important emerging field. As our population ages, tissue engineering and regenerative medicine will become important economic forces, and the United States must be prepared to lead.

ACKNOWLEDGEMENTS

To construct this report the authors have drawn heavily on the presentations and papers of the participants in the WTEC Workshop on Tissue Engineering Research in the United States and of the European and Japanese sites visited by the WTEC team. We are grateful for the hospitality and assistance of all our hosts and colleagues in this endeavor.

REFERENCES

Bonasser, T.J., and C.A. Vacanti. 1998. Tissue engineering: The first decade and beyond. *J. Cell. Biochem. Suppl.* 30/31:297-303.

Galletti, P.M., K.B. Hellman, and R.M. Nerem. 1995. Tissue engineering: From basic science to products: A preface. *Tissue Engineering* 1 (2):147-149.

Hellman, K.B., E. Knight, and C.N. Durfor. 1998. Tissue engineering: Product applications and regulatory issues. In W.C. Patrick, A.G. Mikos, and L.V. McIntire, eds. *Frontiers in tissue engineering*, 341-366. Amsterdam: Elsevier Science.

Hellman, K.B., R.R. Solomon, C. Gaffey, C.N. Durfor, and J.G. Bishop, III. 2000. Tissue engineering: Regulatory considerations. In R. Lanza, R. Langer, and J.P. Vacanti, eds., *Principles of tissue engineering*, 2nd ed., 915-928. San Diego, CA: Academic Press.

Langer, R. and J.P. Vacanti. 1993. Tissue engineering. *Science* 260:920-926.

Lysaght, M.J., and J.A. O'Loughlin. 2000. Demographic scope and economic magnitude of contemporary organ therapies. *ASAIO Journal* 46:515-521.

Lysaght, M.J., and J. Reyes. 2001. The growth of tissue engineering. *Tissue Engineering. 7 (5):485-493.*

Skalak, R. and C.F. Fox, eds. 1988. *Tissue engineering*, Proceedings of a Workshop held at Granlibakken, Lake Tahoe, California, February 26-29, 1988. New York: Alan Liss.

CHAPTER 2

BIOMATERIALS

Linda G. Griffith

INTRODUCTION

Tissue engineering inherently involves recreation of a 3D tissue structure from a source of cells that may be derived from an endogenous source in the patient (e.g., bone wound healing) or from a donor (e.g., skin, in *in vitro* diagnostic applications). Biomaterials are used to guide the organization, growth, and differentiation of cells in the process of forming functional tissue and provide both physical and chemical cues. Tissue-engineering applications can be broadly classified into two categories: *therapeutic applications*, where the tissue is either grown in a patient or grown outside the patient and transplanted; and *diagnostic applications*, where the tissue is made *in vitro* and used for testing drug metabolism and uptake, toxicity, pathogenecity, and so on. The materials requirements for each of these broad categories are distinct but overlapping.

For therapeutic applications, one of the most desirable material properties is degradation or resorption (Griffith 2000). Although some tissues, particularly bone, can tolerate very slowly degrading or permanent materials of specific compositions, permanent implants almost always elicit a chronic inflammation called a foreign body response (Anderson 1988; Babensee, Anderson, et al. 1998; Anderson and Langone 1999). This response is characterized by formation of a poorly vascularized fibrous layer analogous to a scar at the material-tissue interface. Materials and their degradation products must also be nontoxic and non-immunogenic upon implantation. Further aspects of basic biocompatibility are typically context-dependent. For example, lactic and glycolic acid breakdown products produced by degradation of commonly-used degradable polyesters have been associated with adverse tissue reactions when used as fixation devices in bony sites (Böstman, Hirvensalo, et al. 1990; Suganama, Alexander, et al. 1992), presumably due to the rapid release of degradation from relatively large devices.

However, these same polymers, when formulated into structures that are porous and have relatively little polymer-per-unit volume, perform in an acceptable fashion, as is the case with skin regeneration. Degradable synthetic polymers undergo extensive chain scission to form small soluble oligomers or monomers. Degradation may proceed by a biologically active process (e.g., enzymes present in body fluids participate) or by passive hydrolytic cleavage. The term “biodegradable” typically refers to materials in which active biological processes are involved. Resorbable polymers gradually dissolve and are eliminated through the kidneys, metabolism, or other means. Most degradable materials used in tissue engineering today were adapted from other surgical applications, but new polymers specifically designed for tissue engineering are rapidly emerging. New degradable materials with improved mechanical properties, degradation properties, cell-interaction properties, and processability are needed, and development of such materials is an intense area of activity in the field of tissue engineering.

Desirable mechanical properties of biomaterials and devices vary widely with application, and constraints may range from *in vivo* performance needs (e.g., matching tissue compliance) to practical issues of ease of handling in a laboratory or intraoperative setting, where excessively brittle or excessively limp devices may

increase error or failure rates. Obtaining a specific range of mechanical properties is generally of great importance in load-bearing connective tissue applications such as bone, cartilage, and blood vessel replacement.

From a macroscopic perspective, it is the device mechanical properties that matter. Device mechanics are governed both by materials composition and by materials processing methods. For example, the same poly(lactide-co-glycolide) polymer formulation can be made into flexible fabrics (e.g., Vicryl mesh used for Dermagraft™) as well as into rigid solid or porous blocks. In most tissue-engineering devices, mechanical properties of the device itself are not constant because the device is degrading as the tissue grows, and degradation properties can be affected not only by the composition and structure of the material, but also by the mechanical load at the site of use. As more animal and clinical data emerge relating device performance to structure and composition, efforts to better tailor the time-dependent aspects of mechanical properties will increase. In addition to the role of bulk mechanical properties on device performance, the local cell-molecular-level mechanics may also govern tissue response through modulation of cell behavior. The role of such effects requires an iterative approach to build materials that affect cell processes and then assess response.

Biomaterials used in tissue engineering can be broadly divided into categories of *synthetic* or *naturally derived*, with a middle ground of *semisynthetic* materials rapidly emerging. Most materials commonly in use in tissue engineering today—in clinically approved products or in applications at an initial research stage—are adapted from other surgical uses, such as sutures, hemostatic agents, and wound dressings. These include synthetic materials such as polylactide-co-glycolide polymers (component of Dermagraft™) and naturally-derived materials such as collagen (component of Appligraf™). Adaptation of materials that have already been used in other applications in humans can have some advantages from the regulatory perspective, as the safety and toxicity profiles of the materials in humans are already defined. Thus, there can be confidence that materials composition of new devices will be safe; other performance aspects such as cell-material interactions and degradation properties, however, are not assured. This need for substantially higher performance characteristics is pushing research and development in the design of new materials that meet specific performance criteria in tissue engineering.

A particular challenge in addressing materials issues for tissue engineering is that the biological processes are not yet understood well enough to allow a clear set of design parameters to be specified *a priori*. Indeed, evolution of materials/devices and knowledge of biological processes occur simultaneously. New materials/devices illuminate the enormous complexity of biological responses—which then inform the improved design of materials and scaffolds.

It is clear, though, that at the molecular materials design level, there is a substantial need for new materials that interact with cells via highly specific receptor-mediated phenomena, controlling ligation of both adhesion and growth factor receptors and responding to the wound-healing environment by degrading on cue. Design of such materials is proceeding along two parallel paths. The first challenge is to understand quantitatively how cells respond to molecular signals and integrate multiple inputs to generate a given response. This challenge is significant, considering that the number of cellular regulatory molecules identified so far represents only a fraction of the total that exist in the normal tissue environment. Emerging as tools to study these issues are model polymeric and oligomeric systems, synthesized without constraints of *in vivo* biocompatibility or cost, and thus having the potential for very precise control of molecular and supramolecular structure. Model systems are needed to enable systematic investigation of the combined role of physical and chemical aspects of signaling from the extracellular matrix (ECM) and growth factors by controlling the precise density and spatial organization of ligands in the cell environment. For example, much evidence supports the idea that integrin adhesion receptors require aggregation for proper signal generation (Kornberg, Earp, et al. 1991; Miyamoto, Akiyama, et al. 1995). Classical cell biology approaches are generally not amenable to quantitative analysis of this phenomenon. For example, determining how the size and number of integrin receptors aggregate affects not only signaling but downstream responses such as cell growth and migration. Model systems that allow these quantitative, physical issues to be understood thus provide the design basis for clinical implant materials, where design constraints include composition, mechanical properties, stability, processability, and cost.

At the mesoscopic and macroscopic levels, scaffold structures may also be classified as adapted or designed. Fabrics, foams, and even sea coral have been adapted from other applications to serve needs in tissue engineering, providing a first round of information in how device structure influences performance. As the need for performance increases, new approaches to materials processing are required to create scaffolds with complex architectures and macroscopic shapes, and which allow composition variation to accommodate variations in evolving tissue structure. Ultimately, processing approaches must be adaptable to manufacturing protocols that are cost-effective and can meet FDA requirements for good manufacturing processes.

U.S. R&D ACTIVITIES

The United States has a strong research and development effort in adapting existing materials to tissue engineering as well as in design and development of new materials with improved bulk properties and cell-interaction parameters.

Degradable Synthetic Bulk Polymers

Synthetic degradable polyesters were adopted in surgery 30 years ago as materials for sutures and bone fixation devices (Kulkarni, Moore, et al. 1971) and remain among the most widely used synthetic degradable polymers. Degradable polyesters derived from three monomers—lactide, glycolide, and caprolactone—are in common clinical use and are characterized by degradation times ranging from days to years depending on formulation and initial M_w. Johnson & Johnson (Tunc 1991), Davis & Geck (Watts, Carr and Hohf 1976), and other companies developed formulations that have since been adopted for use in tissue engineering. High-quality polymers suitable for human implantation are available from Birmingham Polymers, which also provides custom synthesis (Boehringher Ingelheim competes in Europe). Poly-lactide-co-glycolide polymers are the materials used in a recently approved skin regeneration product, Dermagraft™. The synthesis of these polymers by ring-opening of lactides and glycolides is relatively expensive, resulting in final product costs of $2-5000/kg. Cargill, Inc., is developing a new low-cost synthesis that may reduce the price an order of magnitude or more, although the focus of its products is consumer packaging.

The mechanical properties of the classical degradable polyesters are not always suitable for tissue engineering, due to their relative inflexibility and tendency to crumble upon degradation. This has led to development of additional polymers, notably poly(hydroxybutyrate) (PHB) and co-polymers of hydroxybutyrate with hydroxyvalerate (Amass, Amass, et al. 1998). Tepha, Inc., a spin-off of Metabolix, is developing poly-4-hydroxybutyrate for tissue-engineering applications. These polymers are produced in microorganisms and processed post-purification; they are included in this category due to their chemical simplicity and similarity to classical degradable polymers.

The acidic degradation products of the classical polyesters PLA, PGA, PCL, and their copolymers, have been implicated in adverse tissue reactions, particularly in bony sites. These limitations are being addressed by synthesis of polymers that yield less acidic degradation products and yet still have suitable strength and degradation properties. Researchers at Rutgers have pioneered new families of polymers; for example, Kohn and colleagues have developed materials based on tyrosine carbonates, which are well-tolerated in bony sites (James and Kohn 1996). Formulations have been licensed by Integra, Inc. (a Johnson & Johnson company), and by a start-up company, Advanced Materials Design (NY City). Researchers at Rice University and the University of Colorado are also developing new bulk polymers targeted primarily to bony applications (Anseth, Shastri, et al. 1999). Work at MIT by Langer and colleagues has led to many new bulk synthetic polymer formulations designed primarily for drug delivery, but these are being explored for tissue engineering as well.

Synthetic Gels

The use of synthetic gels is emerging primarily as a way to deliver cells or scaffolds *in situ*. A predominant approach, pioneered by Hubbell, is formation of photopolymerizable gels using PEO-based substrates (Han and Hubbell 1997). This technology formed the basis of a start-up, Focal, Inc., recently purchased by Genzyme. Langer and coworkers at MIT have pioneered a process of forming a gel by shining light through

the skin on injected monomers to form a gel, providing a means for improved minimally invasive delivery (Elisseeff, Anseth, et al. 1999). This approach may be particularly useful for applications such as "injectible cartilage." A chemical gelation approach developed by Harris and coworkers is being developed primarily for drug delivery applications but with potential for tissue engineering as well (Zhao and Harris 1998). These materials have been licensed by a start up company, Confluent, Inc.

Natural Polymers

Natural polymers include both extracellular matrix (ECM) proteins and derivatives (e.g., collagen) and materials derived from plants and seaweed. Type I collagen, the main structural protein in ECM, and mixtures of Type I collagen and other matrix components have been successfully used in several tissue-engineering applications, notably the artificial skin scaffold developed by Yannas (now licensed by Integra Life Sciences and being developed for cartilage and other applications) and the collagen gel process for forming skin implemented by Organogenesis. Collagen Matrix, Inc. (NJ) is implementing a process to extract Type I collagen in native triple helical form with telopeptides intact for a range of uses in tissue engineering. NeuColl has products for the orthopedic market, notably Type I collagen combined with ceramic for bone regeneration. A combined collagen-mineral product for bone regeneration is also being marketed in Europe by California-based Orqest. Concerns about immunogenicity and safety of processed bovine collagen, while slight, have stimulated development of recombinant techniques for producing triple-stranded human collagen for both pharmaceutical as well as surgical and tissue-engineering applications. Fibrogen, Inc. (CA), is a prominent player in this arena. Other ECM components are being developed as well, such as the laminin-family proteins produced by Desmos, Inc., targeted to epithelial tissue engineering, including islets. Fibrin, derived from blood, has also been explored as a matrix.

Matrices for tissue engineering are also being derived by extraction or partial purification of whole tissue, removing some components and leaving much of the 3D matrix structure intact, likely with growth factors as well. Demineralized bone matrices (e.g., Osteotech's Grafton and the recently introduced Exactech product based on human bone) are used clinically in bone wound healing and may be considered a form of tissue engineering matrix. The partially purified small intestinal submucosal matrix, developed initially at Purdue University, has been shown to induce regeneration of a variety of tissues and is under development by Indiana-based Cook Biotech. It currently has a veterinary market and is in research for a number of human applications including blood vessels and ureters, with additional submucosa-type matrices in development at Purdue.

In addition to protein-based materials, there is significant activity in the area of natural polysaccharides. Hyaluronic acid (HA), enriched during wound healing and development, is being developed as a biomaterial by several companies, including Clear Solutions (NY), Genzyme (MA), and Orquest (CA). These companies are targeting a variety of surgical applications with tissue-engineering applications in a second generation. Because HA is water-soluble, it must be cross-linked or otherwise modified to form a scaffold. Alginate, a charged polysaccharide from seaweed that gels in the presence of calcium, has been used in wound healing and *in vitro* cell culture and is being developed for tissue engineering in native and modified forms. When used as a solution for injecting cells, it can form a solid matrix to treat uretogenital disorders, an approach developed at MIT and Harvard and in Phase III clinical trials by Curis, Inc. Alginate has several deficiencies in native form: it is not readily resorbable, and it does not interact with cell surface receptors in any known physiological manner. Mooney and colleagues at the University of Michigan have been tailoring alginate to perform in tissue-engineering applications by using oligomeric forms combined with peptides and other synthetic components to control biological activity, degradation, and mechanical properties.

Synthetic Materials with Tailored Biological Ligands

A major focus of research in the United States is developing materials that control cell behavior via specific receptor-ligand interactions, with some products moving into commercial application. The prototypical adhesion sequence, RGD, was derived from fibronectin by Piersbacher and Rouslhati about 20 years ago and formed the basis for a start-up company, Telios, Inc. (CA). Telios, currently owned by Integra, Inc., focused on both pharmaceutical and tissue-engineering applications and developed a product based on presenting adhesion peptides by incorporating a long hydrophobic tail that enabled strong, near-irreversible adsorption of the peptides to a range of surfaces. The coating was promoted as a means of enhancing tissue ingrowth

and is currently under investigation by Integra as a modification of collagen scaffolding. Since the publication of the original sequence, hundreds of new adhesion sequences from ECM have been identified in the scientific literature, which induce adhesion by all known and many unidentified adhesion receptors. Translation of the discoveries to use on a commercial scale has proceeded slowly, perhaps due to the need for more than one signaling molecule or the need to understand physical and chemical rules of ligand presentation. In addition to Telios/Integra, Protein Polymer Technologies has developed silk protein-based polymers that present ligands. Virtually every academic biomaterials program in the United States includes at least some project on use of tailored adhesion peptides, and much effort is directed at developing model systems that will inform the design of real biomaterials. Notable programs in this arena include U.C. Santa Barbara, Cal Tech, University of Washington, MIT, Georgia Tech., Harvard University, University of Michigan, U.C. Berkeley, Rice University, and Case Western Reserve.

The use of adhesion peptides is also merging with controlled presentation of growth factors as either bound or tethered to the substrate. This remains an area of intense academic research.

Scaffold Technologies for Implantable Devices and Tissues

In addition to chemical composition, the structure of the scaffold plays a role in guiding tissue development. Three very general scaffold types can be delineated: structural scaffolds with an imposed pore structure; gel-type scaffolds formed *in situ* in the presence of cells or tissues; and natural tissue-derived gels (the latter are described above in the sections on gels).

For most tissues, the key requirement that can be defined at present is that interconnected porosity of larger dimensions than the cells is required or desired. A variety of woven and non-woven fiber-based fabrics developed by U.S. surgical companies (Johnson & Johnson, Davis & Geck) were adapted in the early stages of tissue engineering and remain a staple in research programs throughout the United States and abroad; custom production has been available from Albany International. Langer of MIT and Vacanti of Harvard had the first intensive efforts in this area, with a focus on liver and cartilage; they remain strong contributors for a variety of organ systems. Degradable fabrics have been adapted for use in skin (Advanced Tissue Sciences—ATS) and are under intense research and development for bladder (Harvard), blood vessels (MIT, Harvard, Duke), cartilage (U. Mass Medical, Harvard, MIT, ATS), intestine (Harvard) and others. They appear to be particularly useful for layer structures such as skin, intestine, and bladder.

The limitations of fabrics are lack of specific shape and possibly suboptimal microarchitecture. A variety of alternative approaches have been developed in the United States, including freeze-drying, particulate leaching, foaming, and solid free-form fabrication. This work has occurred primarily in academic institutions, with current active programs at virtually every school that has a biomaterials effort (for examples, see the schools listed above for peptide-modified materials). Translation of academic research tools into commercial products has proceeded relatively slowly for a variety of reasons. Fabrication processes must be ultimately carried out in accordance with FDA good manufacturing processes, requiring reproducibility and quality control. Several of the fabrication methodologies employ solvents to achieve the final structure. Residual solvents must be removed to comply with FDA regulations, and solvent removal processes can destroy fine details of architecture. Thus, many of the research scaffolds developed may be most useful for determining the role of architecture on tissue development, while practical applications will require alternative fabrication techniques.

The United States has a reasonably strong effort in developing manufacturing technologies for complex scaffolds, using methods derived from other manufacturing fields, notably solid free-form fabrication (SFF), which involves building solid objects as a series of thin 2D slices, usually using a CAD/CAM program to control the addition of material. The most visible method in this family for use in medicine is stereolithography, which has long been used to make models for surgery and involves photopolymerization of a liquid monomer. Efforts at Princeton and Carnegie Mellon Universities are directed at making devices for bone-tissue engineering. A challenge for this approach is development of appropriate polymerizable monomer systems. An alternative SFF method, the 3DP™ printing process, was developed at MIT and is being commercialized by Therics, Inc. (Princeton, NJ). This method employs printing a liquid binder into a bed of polymer or ceramic powder and can be used to create objects with different compositions. An

advantage of the approach is its application to a very wide variety of materials. The resolution of both the stereolithography and 3DP™ methods are comparable.

JAPANESE R&D ACTIVITIES

Degradable Synthetic Bulk Polymers

Most work in Japan involves off-the-shelf polymers or near-off-the-shelf polymers. For example, at the University of Tsukuba, polylactic capralactone in a foam format is being used for cartilage regeneration.

Synthetic Gels

A novel approach to exploiting gels in tissue engineering is work by Okano and co-workers at Tokyo Women's Medical University . They are using thermal-reversible gels to create cell sheets that can detach and be used for tissue engineering (Kushida, Yamato, et al. 1999). Matsuda's group at Kyushu University is developing photopolymerizable gels based on gelatin linked with styrene monomers, which can then be modified with heparin or other molecules for vascular grafts. These gels are thus semisynthetic.

Natural Polymers

Kyoto University Institute for Frontier Medical Sciences has a large effort in several aspects of naturally derived materials. The scaffolds being developed consist of extracellular matrix obtained by complete removal of cell components from allogeneic or heterogeneic organs or tissue. The de-cellularized matrix is mixed with reconstituted collagen types I, III, and IV, extracted from swine skin by enzyme treatment in a neutral solution to abolish immunogenicity. For reinforcement, the extracellular matrix is combined with synthetic biodegradable polymers. In some cases, cells and/or growth factors are added. Target tissues and organs include the following:

1. membranes, such as the cornea, pericardium, pleura, peritoneum, and dura matter of the brain
2. tubular organs, such as blood vessels, trachea, and digestive tubes
3. tissues receiving external force, such as teeth, periodontal membrane, cartilage, bone, tendons, and ligaments
4. neurological systems, such as the peripheral nerves and spinal cord
5. urological systems, such as the bladder and ureters
6. parenchymal organs, such as the lungs, heart, liver, and kidneys

For the pericardium, pleura, and dura matter of the brain, membrane sandwiches made of collagen and PGA and coated with gelatin are used. Work with natural collagen, following the work of Howard Green in the United States, has been the focus of skin tissue engineering at Nagoya University. Work at the National Cancer Center Institute in Tokyo focuses on atelocollagen as a gene-delivery matrix.

Menicon, Inc., in Nagoya has a primary focus on contact lenses and eye care but has developed a process for expansion of skin using scaffolds similar to those developed by Yannas.

Synthetic Materials with Tailored Biological Ligands

There is modest activity and there are few focused efforts in Japan on synthetic materials with tailored biological ligands. Akaike and colleagues at Kanagawa Institute of Technology (KAST)/Tokyo Institute of Technology have a strong program developing polymers for liver tissue engineering, synthesizing materials that target the asialoglycoprotein receptor and other cell surface ligands (Cho, Goto, et al. 1996). This lab is fairly strong at the science/engineering interface and has several collaborations within Japan and Korea. Ito and Imanashi have worked for several years to develop materials that present specific biological ligands such as insulin (Zheng, Ito, et al. 1994).

Scaffold Technologies for Implantable Devices and Tissues

Most efforts focus on using scaffolds that are available, but focused efforts are underway to develop new processes. Investigators at Hokkaido are using casting techniques to make 2D degradable polymer scaffolds for liver tissue engineering with 10 micron resolution, and then combining them to make 3D structures. Matsuda's group at Kyushu has a strong effort in construction of cardiovascular devices, and his group includes 2D patterning as well as laser-based 3D fabrication techniques with an emphasis on cardiovascular applications.

EUROPEAN R&D ACTIVITIES

Degradable Synthetic Bulk Polymers

Several efforts are underway throughout Europe to adapt existing polymers and to produce improved degradation and mechanical properties. Sittinger and colleagues in Berlin (Charite Hospital/Humbolt University) are adapting degradable polyester fabrics for cartilage regeneration (Perka, Sittinger, et al. 2000), following work of Freed et al. and ATS et al. in the United States (Vunjak-Novakovic et al. 1999). A group at the German Heart Institute in Berlin is using technology developed in Boston by the Mayer group to create heart valves based on degradable polyesters, including poly-4-hydroxybutyrate from Tepha (U.S. company). Hocker and colleagues at the University of Technology in Aachen are synthesizing new bulk polymers built from alternating lactic acid and amino acid monomers to lessen acidic effects on degradation and move toward more erosive (rather than bulk degradation) properties. The center of competence established at Aachen has extensive experience with taking cardiovascular materials into clinical trials and is expecting to spin off a start-up company based on technology developed at the center within 3 years. Also addressing the relatively poor mechanical and degradation properties of classical polyesters, the Suter group at ETH in Zurich has synthesized block copolymers with polyurethane linkages, allowing a far greater range of mechanical properties to be achieved (Hirt, Neuenschwander, et al. 1996; Saad, Matter, et al. 1996). These multiblock copolymers have crystallizable hard segments of PHB and non-crystallizing oligoesters (adipic acid, ethylene glycol, 1,4-butanediol, and diol-terminated PCL) as soft segments. The strong group at the University of Twente (Feijen et al.) is building materials with trimethylene carbonate and caprolactone (Pego, Poot, et al. 2001). The group at the University of Nottingham led by Downes (Smith and Nephew/Nottingham U.) is adapting composites of degradable polyesters with hydroxyapatite for orthopedic applications.

Synthetic Gels

A degradable copolymer of PEO and PBT that forms a hydrogel with properties that can be modulated by the relative ratios of the two contributing monomers and termed Polyactive, is being investigated at a number of sites for applications ranging from skin to bone. The company Isotis (Netherlands) is developing Polyactive for a range of applications. Founders have reported good bone bonding in animal studies (Sakkers, Dalmeyer, et al. 2000), but it has recently been reported to be a poor bone-bonding material in humans in a study by a group in Marburg (Roessler, Wilke, et al. 2000). Extensive work by Hubbell's group at ETH (Han and Hubbell 1996) is noted under the section on bioactive materials below entitled, "Synthetic Materials with Tailored Biological Ligands."

Natural Polymers

A strong program integrating molecular biology with matrix design exists at the University of Manchester, where the biomaterials group is well integrated with researchers at Matrix Biology; they have, for example, produced novel collagen mutants in the milk of mice. The integrated groups in the upper Rhine Valley at the Valley Tissue Engineering Center are primarily surgeons who use existing collagen and fibrin materials for applications in skin and bone. In Berlin (Humboldt University/Charite Hospital), chitosan/gelatin hydrogels are being developed for tracheal epithelia, and fibrin matrix is being explored for other applications. Hubbell's group at ETH has made significant advances modifying fibrin with additional biological ligands (Herbert, Nagaswami et al. 1998). Fidia, Inc. (Italy) has developed a series of modified hyaluronate esters, adding hydrophobic moieties to the carboxyl groups (Iannace, Ambrosio, et al. 1992), to control degradation.

Fidia is currently marketing these as tissue-engineering polymers; they are being applied to growth of bone and cartilage by the group at Padova University (Radice, Brun, et al. 2000).

Synthetic Materials with Tailored Biological Ligands

The premier group working on synthetic materials with tailored biological ligands is Hubbell's at ETH, which is developing materials with biological adhesion sites, growth factors, and degradation sites, and translating these discoveries into technologies, culminating 15 years of work. Applications range from connective tissues (cartilage and bone), to nerve and cardiovascular applications. The group at INSERM (Bordeaux) is employing a variety of model systems to understand fundamentals of cell interactions with adhesion peptides and to parse the processes of tissue integration and inflammation. It is strongly focused on using bioactive ligands to induce specific cell functions.

Scaffold Technologies for Implantable Devices and Tissues

Many of the scaffold technology methods in Europe are comparable to those in the United States, for example, production of porous nerve guides by immersion precipitation (Pego, Poot, et al. 2001). A group at INSERM in Nancy is employing stereolithography for making ceramic scaffolds for bone tissue engineering. Solid free-form fabrication methods with relatively low resolution (1-2 mm, compared to 0.3-0.8 mm for U.S. methods) are being used by the group in the German Heart Institute in Berlin. Fidia is making porous scaffolds for skin regeneration.

SUMMARY

The United States pushed the initial development of approaches based on adapting surgical polymers (degradable polyesters, collagen matrices) and scaffolds for use in tissue engineering, and this approach has rapidly been adapted in Europe and especially in Japan. In the United States, large academic programs exist at several universities, funded by a combination of the National Institutes of Health (NIH) (RO1, PO1, and BRP grants) and the National Science Foundation (NSF) (single investigator programs plus engineering research centers located at the University of Washington, Georgia Tech, and MIT), as well as by industry. The centers of large-scale activity include Cal Tech, Case Western Reserve, Georgia Tech, MIT, Rice, University of Michigan, Rutgers, U.C. Santa Barbara, and University of Washington. Many other schools have active investigators as well. The funding of the University of Washington Engineered Biomaterials Engineering Research Center (ERC), and that of linked programs at Georgia Tech's ERC for Engineered Tissues and MIT's Biotechnology Process Engineering Center (which focuses on gene delivery and stem cells), totals ~$5 million/year. This has increased the visibility and coordination of academic research in biomaterials; further, the requirement that these centers work with industrial advisory boards has fostered strong industry-academic interactions in the United States. Initiation of the BRP program by NIH, with substantial participation by the National Heart, Lung and Blood Institute, has also stimulated significant research in biomaterials and fostered industry interactions.

Large companies with existing surgical materials programs, such as Johnson & Johnson, have moved into tissue engineering primarily through acquisition of new technology from academia and purchase of small companies rather than through in-house development (for example, Integra purchased Telios, and is now a Johnson & Johnson company). Formation of " the Corporate Biomaterials" division at Johnson & Johnsonis one signal of industry's major new focus of attention on this field. The NIST ATP program has also been a strong supporter of biomaterials and has also served to greatly stimulate academic-industry interactions.

Activity in both Europe and Japan in the general area of tissue engineering is increasing due to an increased level of government funding. The funding is often directed at building interdisciplinary centers of competence such as at Tsukuba and Manchester and often has incentives or requirements for technology transfer. In Europe, but less so in Japan, technology is being transferred to industry, often through start-up companies associated with academic research centers. In both regions, the greatest proportion of new effort appears to be devoted to challenges in cell technologies, often in combining cells with existing materials in clinically (i.e., medicine-) driven approaches to regenerating tissues. The issue of cell sourcing is indeed a critical challenge in tissue engineering. Japan, for example, is initiating a central center in Kansai to provide

cells nationally. Many of the tissue-engineering programs employ off-the-shelf biomaterials with the aim of creating novelty through application of cells; they thus do not explicitly focus on development of new biomaterials or even on significant modification of existing biomaterials.

Europe has long had many excellent programs in biomaterials, covering all aspects of natural and synthetic materials, polymers, and ceramics, and these appear to have been bolstered by the new funding for tissue engineering. There continues to be significant activity in developing new bulk biomaterials with novel monomers and strategies for degradation. There is also a complementary level of activity in employing modifications of biologically derived polymers to improve function via addition of specific ligands for cell interactions. A common theme in much of the biomaterials research in Europe is a focus on molecular design principles, including fairly sophisticated knowledge of receptor-mediated cell interactions. In addition to materials synthesis, Europe has many efforts underway in novel materials processing to create scaffolds for tissue engineering.

Japan also has several centers of excellence in biomaterials, and several academic labs are actively developing new materials, including materials that interact with cells via receptor-mediated phenomena. Compared to Europe, however, there is relatively less effort in this direction than on efforts employing existing biomaterials that may be already available off-the-shelf or that can be processed to create structures suitable for tissue engineering. There may be more conservatism in Japan toward commercialization of new materials compositions, particularly since commercialization is usually through large existing companies rather than start-ups.

The field of biomaterials and scaffolds for tissue engineering is in an adolescent phase and maturing rapidly. One of the most significant changes coming in the field is the strong need to integrate basic polymer science and engineering with molecular cell biology and stem cell biology in the design of new materials that carry out very sophisticated signaling needs. Currently, the United States holds the lead in interdisciplinary approaches, with many interdepartmental academic programs at all tiers of the education system. Some focused areas of excellence in integrated research are found in Europe (INSERM, ETH, Lausanne, Manchester, Nottingham), and fewer in Japan (Tokyo Women's Medical University and KAST). Boundaries between disciplines appear less fluid in Japan.

There is a strong need to continue to push integration of basic materials science with the extremely rapid advances in biology that contribute to regenerative medicine. Often, researchers in basic biology and medicine do not have a clear perspective on the essential role of biomaterials in effecting ultimate clinical application, or they are not aware of the long lead times needed to develop effective materials strategies. Likewise, biomaterials researchers may work on problems headed for obsolescence as a result of advances in basic biology. Ultimately, biology is the link between materials science and medicine, required for long-term success in tissue engineering. Although many individual research programs have developed strong, effective, interdisciplinary links, the field is now poised for advances in education to train the next generation of research scientists and engineers. There is an especially critical need to attract more excellent life scientists into the field. The United States is playing a leading role in defining the emerging field of bioengineering from an educational perspective, and leading academic centers in Europe and Japan are following similar approaches. NSF and NIH recently held a joint workshop on Bioengineering, Biomedical Engineering, and Bioinformatics training and education in which strategies for increasing the interdisciplinary approaches were posed. These strategies apply to the field of biomaterials and tissue engineering, and emphasis must be placed on the infrastructure to move the field forward.

REFERENCES

Amass, W., A. Amass, et al. 1998. A review of biodegradable polymers: uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. *Polymer Int.* 47:89-144.

Anderson, J.M. 1988. Inflammatory response to implants. *Trans. Am. Soc. Art. Int. Organs* 34:101-107.

Anderson, J.M. and J.J. Langone. 1999. Issues and perspectives on the biocompatibility and immunotoxicity evaluation of implanted controlled release systems. *Journal of Controlled Release* 57 (2):107-113.

Anseth, K.S., V.R. Shastri, et al. 1999. Photopolymerizable degradable polyanhydrides with osteocompatibility. *Nature Biotechnology* 17 (2):156-159.

Babensee, J.E., J.M. Anderson, et al. 1998. Host response to tissue engineered devices. *Advanced Drug Delivery Reviews* 33 (1-2):111-139.

Böstman, O., E. Hirvensalo, et al. 1990. Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. *J. Bone Joint Surg.* 72-B:592-596.

Cho, C.S., M. Goto, et al. 1996. Effect of ligand orientation on hepatocyte attachment onto the poly(N-p-vinylbenzyl-o-beta-D-galactopyranosyl-D-gluconamide) as a model ligand of asialoglycoprotein. *J. Biomater. Sci. Polym. Ed.* 7:1097-1104.

Elisseeff, J., K. Anseth, et al. 1999. Transdermal photopolymerization for minimally invasive implantation. *Proceedings of the National Academy of Sciences of the United States of America* 96 (6):3104-3107.

Griffith, L.G. 2000. Polymeric Biomaterials. *Acta Mater.* 48:263-277.

Han, D.K. and J.A. Hubbell. 1996. Lactide-based poly(ethylene glycol) polymer networks for scaffolds in tissue engineering. *Macromolecules* 29 (15):5233-5235.

———. 1997. Synthesis of polymer network scaffolds from L-lactide and poly(ethylene glycol) and their interaction with cells. *Macromolecules* 30 (20):6077-6083.

Herbert, C.B., C. Nagaswami, et al. 1998. Effects of fibrin micromorphology on neurite growth from dorsal root ganglia cultured in three-dimensional fibrin gels. *Journal of Biomedical Materials Research* 40 (4):551-559.

Hirt, T.D., P. Neuenschwander, et al. 1996. Synthesis of degradable, biocompatible, tough block co-polyesterurethanes. *Macrmol. Chem. Phys.* 197:4253-4268.

Iannace, S., L. Ambrosio, et al. 1992. Thermomechanical properties of hyaluronic acid-derived products. *J. Mat. Sci: Mat. in Med.* 3:59-64.

James, K. and J. Kohn. 1996. Applications of pseudo-poly(amino acid) biomaterials. *Trends in Polymer Science* 4 (12):394-397.

Kornberg, L.J., H.S. Earp, et al. 1991. Signal transduction by integrins: Increased protein tyrosine phosphorylation caused by clustering of beta-1 integrins. *Proc. Nat. Acad. Sci. USA* 88:8392-8396.

Kulkarni, R.K., E.G. Moore, et al. 1971. Biodegradable poly(lactic acid) polymers. *J. Biomed. Mat. Res.* 5:169-181.

Kushida, A., M. Yamato, et al. 1999. Decrease in culture temperature releases monolayer endothelial cell sheets together with deposited fibronectin matrix from temperature-responsive culture surfaces. *Journal of Biomedical Materials Research* 45 (4):355-362.

Miyamoto, S., S.K. Akiyama, et al. 1995. Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. *Science* 267:883.

Pego, A.P., A.A. Poot, et al. 2001. Copolymers of trimethylene carbonate and epsilon-caprolactone for porous nerve guides: synthesis and properties. *J. Biomater. Sci. Polym. Ed.* 12:35-53.

Perka, C., M. Sittinger, et al. 2000. Tissue engineered cartilage repair using cryopreserved and noncryopreserved chondrocytes. *Clin. Orthop.* 378:245-254.

Radice, M., P. Brun, et al. 2000. Hyaluronan-based biopolymers as delivery vehicles for bone-marrow-derived mesenchymal progenitors. *J. Biomed. Mat. Res.* 50:101-113.

Roessler, M., A. Wilke, et al. 2000. Missing osteoconductive effect of a resorbable PEO/PBT copolymer in human bone defects: a clinically relevant pilot study with contrary results to previous animal studies. *J. Biomed. Mater. Res.* 53:167-173.

Saad, B., S. Matter, et al. 1996. Interactions of osteoblasts and macrophages with biodegradable and highly porous polyesterurethane foam and its degradation products. *J. Bioemd. Mat. Res.* 32:355-366.

Sakkers, R.J., R.A. Dalmeyer, et al. 2000. Use of bone-bonding hydrogel copolymers in bone: An in vitro and in vivo study of expanding PEO-PBT copolymers in goat femora. *J. Biomed. Mat. Res.* 49:312-318.

Suganama, J., H. Alexander, et al. 1992. Biologic response of intramedullary bone to poly-l-lactic acid. In *Tissue-Inducing Biomaterials*. L.G. Cima (ed.). Pittsburgh, Materials Research Society. 252:339-343.

Tunc, D.C. 1991. Body-absorbable osteosynthesis devices. *Clin. Mater.* 8(1-2):119-23.

Vunjak-Novakovic, G., I. Martin, B. Obradovic, S. Treppo, A.J. Grodzinsky, R. Langer, and L.E. Freed. 1999. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *J. Orthop. Res.* Jan;17(1):130-8.

Watts, D.R., S.H. Carr, and R.P. Hohf. 1976. Poly(glycolic acid) sutures in canine vascular anastomoses. *J. Biomed. Mater. Res.* Nov;10(6):867-77.

Zhao, X. and J.M. Harris. 1998. Novel degradable poly(ethylene glycol) hydrogels for controlled release of protein. *J. Pharm. Sci.* 87:1450-1458.

Zheng, J., Y. Ito, et al. 1994. Cell growth on immobilized cell-growth factor. *Biomaterials* 15 (12):963-968.

CHAPTER 3

CELLS

Nancy L. Parenteau

INTRODUCTION

Cells are the functional elements of repair and regeneration. Successful tissue engineering hinges on the ability to

1. accurately predict cell response
2. acquire the appropriate cells
3. cultivate the cells for proliferation and cell differentiation to an appropriate phenotype or function

This assessment cites a number of references for illustrative purposes, to reflect the type of work being done or highlight the progress being made. The bibliography is by no means all-inclusive.

ANALYSIS OF CURRENT STATE OF THE ART AND FACTORS INFLUENCING PROGRESS

Ability to predict cell response

Accurate prediction of cell response relies on an adequate level of understanding in cell biology, extracellular matrix biology, developmental biology, and physiology, as well as immunology and inflammation. This fundamental knowledge is essential to effective design in tissue engineering, whether the goal is the development of a novel scaffold to promote tissue regeneration or the development of a living cellular implant. Without it, tissue engineering is practiced in a proverbial black box, using an iterative approach often lacking the dimension and understanding needed to produce a successful, predictable outcome in a timely manner. To be competitive, tissue engineering must incorporate principles of biology.

There are several relatively new analytical tools that will play important roles. Polymerase chain reaction (PCR) analysis and gene array technology will allow in-depth study of gene expression. This will be used to characterize cellular phenotype and understand cause and affect relationships at the genetic level (Shamblott et al. 2001). Protein chip technology will enable rapid identification and screening of functional parameters, novel cellular markers (phenomics), and autocrine and paracrine factors influencing cell populations. It has the potential to reduce to the time period of a few hours what would take days to weeks to accomplish using standard laboratory procedures. U.S. companies Affimatrix and Ciphergen are leaders in gene array and protein chip technologies, respectively. In addition, advanced imaging systems will allow researchers to more accurately assess structural parameters, observe changes, and validate outcomes. Informatics at the gene, cell, and tissue levels will play a critical role in enabling the prediction and control of cell response. An overview and analysis of the U.S. competitive position in bioinformatics is presented in Chapter 7.

Identification of markers of cell lineage differentiation was advanced by research in the area of hematopoiesis (Koller and Palsson 1993). From this, researchers derived an understanding of stem cells,

their progeny, and the generation of diverse, functional cell populations (Weissman 2000). Much less is known regarding the generation of diverse cell populations in other cell systems (Fuchs and Segre 2000). This knowledge is now being accumulated at a rapid pace, spurred on by the recognized potential of stem cells as a source of cells for repair and regeneration. There are markers being characterized for embryonic stem (ES) cell lineages (Shamblott et al. 2001), as well as adult mesenchymal (Haynesworth, Baber, and Caplan 1992), neural (Uchida et al. 2000), and hepatic cell lineages (Brill et al. 1993). Work of this type is critical for the identification, selection, and control of cell populations for tissue engineering.

Ability to Acquire the Appropriate Cells

One approach to tissue engineering is to provide scaffolds or engineered biomaterials to promote cellular ingrowth and subsequent remodeling into a suitable organ construct. Such materials may be natural polymers such as collagen (Cavallaro, Kemp, and Kraus 1994; Badylak et al. 1999) or synthetic, resorbable materials (Cima et al. 1991). The majority of work involving *in situ* recruitment of cells using scaffolds to regenerate tissue structure has been done using collagen, including the use of processed cadaver dermis (Livesey et al. 1995) or collagen sponge materials to promote dermal regeneration (Heimbach et al. 1988; Yannas et al. 1989); use of collagen to promote the formation of a living blood vessel (Lantz et al. 1993; Huynh et al. 1999); use of native collagen as a scaffold for bladder wall repair (Kropp et al. 1996; Badylak et al. 1998); and use of a collagen prosthesis for tendon repair (Kato et al. 1991). A complete discussion of biomaterials is given in Chapter 2, and a discussion of a biomaterial's ability to promote selective cell ingrowth is given in Chapter 6.

Cell Sourcing

Cell sourcing is a key element enabling or prohibiting potential applications in tissue engineering. There are a variety of choices, depending on the application:

1. autologous cells (the host's own cells)
2. allogeneic cells (cells from a donor)
3. xenogeneic cells (cells from a different species)
4. immortalized cell lines, either allogeneic or xenogeneic
5. stem cells, either allogeneic (fetal or adult derived) or autologous, (adult derived)

The choice of cell source influences many design parameters, such as culture requirements and delivery strategies (Young et al. 1997). It also will influence time to clinical implementation, government regulation, and commercial strategy (Ratner 2000). The use of autologous cells is often seen as the most obvious and expedient route to clinical application of a tissue-engineered product, due to the reduced regulatory and safety requirements compared to the use of allogeneic or xenogeneic cells. It is often assumed that use of autologous cells implies minimal manipulation and maximum safety for the host because of use of the host's own cells. This is not entirely correct, as culture processes and reagents can alter cells regardless of their origin. The use of autologous cells, while enabling expedient clinical use, often delays demonstration of true clinical benefit because of the reduced pressure to show efficacy in a controlled clinical trial, something required when using a non-autologous cell source.

The lack of an up-front test of efficacy, combined with the inherent limitations of using an "individualized" cell source can inhibit or altogether prohibit the incorporation of more effective design parameters. This can, in some instances, actually slow progress of a truly effective therapy rather than enable it. Significant differences in regulatory requirements, while still quite prevalent in Europe, are lessening in the United States. The commercial implementation, proof of efficacy, and commercialization of autologous cell products is now under regulation in the United States. This is primarily to ensure safety of processes, although tracking of efficacy is also now being requested. Regulation of tissue engineered products is covered in detail in Chapter 9; however it is clear that choice of cell source has impact on a technology beyond issues of immunology, safety, and "time to market."

The use of allogeneic and xenogeneic sources present unique immunological and safety considerations. Once past the immunological issues, the use of allogeneic cells should be biologically identical to the use of autologous cells. A close examination of the immunology is needed to determine immune reactivity, since biological reasons for persistence are likely to be the same whether an autologous or allogeneic source is

used, provided the cells are from a similar source and are treated similarly. Another important aspect to conflicting results in the literature regarding the ability to use allogeneic cells lies in the purity of cell populations. While the cell of import may be non-immunogenic, passenger lymphocytes, endothelial cells, dendritic cells, and others still carried in the cell culture population could give rise to sensitization against alloantigens. Therefore, ability to culture only the desired cell types is important for the implementation of some allogeneic cell therapies.

There is now substantial accumulated clinical experience regarding the lack of immunogenicity of an allogeneic skin construct consisting of epidermal keratinocytes and dermal fibroblasts (Falanga et al. 1998; Joseph Laning and Janet Hardin-Young, personal communication, 2001). *In vivo* studies using humanized immunodeficient (SCID) mice have validated the lack of a T cell response (Briscoe et al. 1999). *In vitro* studies confirm the inability of the keratinocyte or fibroblast to elicit a cell-mediated immune response, even in the presence of cytokines known to stimulate T cell response (Laning, DeLuca, and Hardin-Young 1999). Surprisingly, it appears that even experimental sensitization with alloantigen is not sufficient to elicit a cell-mediated response to the allogeneic keratinocytes or fibroblasts (Laning, personal communication). Therefore, certain nonprofessional antigen-presenting cells in the body do not elicit immune response, most likely due to a deficiency in the co-stimulatory pathway of T cell activation (Laning et al. 1999). Recent work on the characterization of adult-derived hepatocyte progenitor cells also suggests that some cell populations may lack even Major Histocompatibility Class I antigens (Kubota and Reid 2000). These data present the possibility of using many allogeneic parenchymal cell types for tissue engineering. In cases where the cells *are* professional antigen-presenting cells (e.g., endothelial cells), there are innovative approaches being developed to block the costimulatory pathway of T cell activation (Larsen et al. 1996; Pearson et al. 1997; Durham et al. 2000).

The use of xenogeneic cells has been viewed as an important alternative in the problem of cell sourcing. Xenogeneic hepatocytes are incorporated in extracorporeal liver assist devices, designed with membrane separation between patient plasma and the porcine cells (Bornemann, Smith, and Gerlach 1996; Catapano et al. 1996; Gerlach 1996). There are also numerous methods of immune isolation involving gel encapsulation of cell aggregates, microencapsulation of cells, and conformational coating of cell clusters (Uludag, De Vos, and Tresco 2000). The challenges of a physical barrier approach lie in the development of suitable biomaterials that are nonreactive and allow adequate oxygenation, free exchange of nutrients, and selective exchange of proteins. Discussions of bioengineering and the modeling of parameters are presented in Chapter 6.

A molecular approach to blocking rejection of xenogeneic cells has been made through genetic manipulation of donor animals to reduce aspects of acute and chronic rejection (Platt 1998; Lee et al. 2000). The hope is to engineer animal organs that will be accepted *in toto* in the human (O'Connell, Cunningham, and d'Apice 2000). This is a challenging alternative approach to generate organs for transplantation and could compete with some applications of tissue engineering where whole organ replacement is warranted. Alternatively, cells from genetically modified animals might serve as source material for tissue engineering and cell therapy approaches (McKenzie and Sandrin 2001). A novel approach to using xenogeneic cells, discovered by U.S. researchers, is to co-culture the cell of interest with testis-derived sertoli cells to confer immune privilege (Platt 1998; Sanberg et al. 1997). This has been proposed for both islet transplantation and neural cell implants. The use of xenogeneic cells also opens the possibility of using fetal tissue from animals where beneficial, such as in the treatment of Parkinson's Disease (Widner 1999) without the obvious ethical concerns and limitations that use of a human source would present. However this must be balanced with the risks associated with the possible transmission of animal viruses. Closed, inbred herds are currently used to control this possibility.

The use of immortalized cells has been limited to date. The principle applications are in the supply of cells for extracorporeal liver assist devices (Ellis et al.1996; Wang et al. 1998) and in the genetic manipulation of beta cells and other cells to create insulin-producing cell lines for treatment of diabetes (Newgard et al. 1999; Cheung et al. 2000). Stem cell technology may obviate the need for some of these approaches as scientists become more experienced in the cultivation of multiple cell types.

Stem cells have the potential to revolutionize cell therapy and tissue engineering. There has been a great deal of both interest and concern over the use of human embryonic stem cells. The ability to cultivate ES cells,

combined with their potential to give rise to virtually all cell types, has opened the door to the possible generation of almost limitless cell sources for a variety of tissues. While this is the far-reaching hope of this technology, it is limited by ethical concerns, and to date, by researchers' rudimentary ability to control or direct cell response.

An alternative is the identification of potential multipotent progenitor cells in adult organs. The discovery and demonstration of multipotent (Kondo and Raff 2000; Oshima et al. 2001), pluripotent (Lagasse et al. 2000), and even totipotent cells (Clarke et al. 2000) in the human adult has given rise to exciting possibilities as a source of cells for cell therapy and tissue engineering (Weissman 2000a). In contrast to ES cells, the challenges are in the identification and isolation of progenitors among the complex array of cells types in the tissue, in the targeted stimulation of their proliferation, and then in the differentiation of the cell toward a functional phenotype.

CONTROL OF CELL PROLIFERATION AND DIFFERENTIATION

The ability to control cell proliferation and differentiation is, at this time, one of the most limiting but important aspects of cellular tissue engineering. Technical knowledge and skill must develop in this area if tissue engineering is to become a successful reality (Bilbo et al. 1993; Parenteau 2000).

Stability of cell phenotype remains a concern, and the science to efficiently direct ES cells to a specific, functional phenotype is still rudimentary. Growth factor response continues to be characterized. A collaboration between U.S. and Israeli researchers (Schuldiner et al. 2000) has characterized the effect of eight growth factors on the differentiation of human embryonic stem cells from aggregates. The researchers divided the effects observed into three categories: (1) growth factors that favored the differentiation of mesodermal cells (Activin-A and transforming growth factor beta); (2) factors that activated ectodermal and mesodermal markers (retinoic acid, epidermal growth factor, bone morphogenic protein-4, and basic fibroblast growth factor); and (3) factors that allowed differentiation of all three embryonic germ layers—ectoderm, mesoderm, and endoderm (nerve growth factor and hepatocyte growth factor)—based on the expression of cellular markers of differentiation. This demonstrated that specific factors favor certain cell lineages, primarily through an inhibition of certain lineages rather than promotion of a specific one. The mechanisms directing *specific* cell lineage are complex. This should not be surprising to anyone versed in developmental biology. However it is a step toward gaining control of what was (i.e., development of the three germ layers), until this point, considered a "spontaneous" event.

Identification and proliferation of progenitor cells from adult organs has led to rapid progress worldwide in the last two years, as exemplified in Table 3.1, through scientific contributions in all regions. As the field progresses there will be an increasing need for development of defined culture systems (Block et al. 1996; Brannen and Sugaya 2000) and permissive environments (Zangani et al. 1999) to not only promote proliferation but as importantly, promote true differentiation and organotypic properties (Parenteau et al. 1992; Zieske et al. 1994).

U.S. R&D ACTIVITIES

The United States gave birth to the field of tissue engineering through pioneering efforts in cell therapy and biomaterials engineering. The United States was also aided by the presence of a strong private sector and entrepreneurial spirit. Over the last decade, tissue engineering has been defined in the United States by activities in biomaterials, bioengineering, and research by physician scientists. This has resulted in a prevalence of work involving the design and use of resorbable biomaterials to promote tissue regeneration, which is covered in Chapter 2. Separately, scientific progress in the field of cell transplantation, cell encapsulation, and extracorporeal devices has been championed by a seemingly unrelated group of researchers.

Growth in the U.S. biotechnology industry led to establishment of several of the first cellular tissue engineering and cell therapy companies around the mid- to late-1980s. Some of the first companies included Marrow-Tech (now Advanced Tissue Sciences), Biohybrid, Biosurface Technologies (Genzyme Tissue

Repair, now Genzyme Biosurgery), Cytotherapeutics (now Stem Cells), Grace Biomedical (now Circe), Hanna Biologics, Neomorphics (now part of Advanced Tissue Sciences), Organogenesis, and Systemix (acquired by Novartis Pharmaceuticals).

BACKGROUND

Despite private and public sector activity over the last 15 years, U.S. progress in the field has been slow. The draw of the biotech industry, which was robust in the late 1980s and early 1990s, combined with increasing competition for government funding, prompted academic researchers to leave the academic bench to start companies to develop a product. The long time lines needed for development of a cell therapy or living tissue therapy taxed entrepreneurial resources. Many good ideas either languished or were relinquished in favor of more expedient but less than robust product strategies. To help offset the risks of pursuing cutting edge technology, the National Institute of Standards and Technology's (NIST) Advanced Technology Program (ATP) has provided funds to U.S. companies to support more ground-breaking strategies such as DNA array technology. The ATP program has actively funded several grants in tissue engineering; however this provides relatively short-term funding (3 years) that must be matched and eventually assumed by an industry partner or the private sector.

Some of the few products of cellular tissue engineering have developed in the area of skin and cartilage: autologous cultured epidermal sheet grafts for burn victims, autologous cultured cartilage cells for articular cartilage repair (both products of Genzyme Biosurgery), and the allogeneic living skin equivalent (Apligraf®[8], Organogenesis, Inc.) for the treatment of chronic diabetic and venous ulcers. Also, in a different approach, Aastrom Biosciences provides a machine to process and cultivate autologous bone marrow cells to enrich for progenitor cells. Although enrichment of lymphocyte populations is one of the areas of cellular tissue engineering that showed early progress, several approaches involving enrichment of specific lymphocyte populations are either still in development or have been discontinued.

Academic efforts in tissue engineering grew through funding from the National Science Foundation and the Whittaker Foundation, which provided several grants to leading university bioengineering departments. Because of this, the engineering activities in tissue engineering grew and remained strong while the activities focused on biological aspects remained weak by comparison, despite a leading position in some specific areas. The WTEC study sought to determine whether and how this has changed.

CURRENT EFFORTS

Research activity in cellular tissue engineering and cell therapy has been dramatically stimulated by the perceived potential of stem cells to impact this area. An analysis of the literature of the last two years indicates that stem cell research is active and competitive worldwide. The United States continues to show strengths in the field of hematopoietic stem cell research (Lagasse et al. 2000; Yagi et al. 1999; Petersen et al. 1999); the differentiation (Pittenger et al. 1999) and clinical use of mesenchymal stem cells (Osiris Therapeutics); analysis and cultivation of hepatocytes (Kubota and Reid 2000); and arguably, embryonic stem cell research (Shamblott et al. 2001). The United States is also active in the commercial development of neural cell transplantation (Diacrin, Stem Cells, Neronyx, Layton Bioscience) and neural stem cell research (Brannen and Sugaya 2000). However, progress by groups in Sweden, Italy, the United Kingdom, and Australia has been equally significant (Table 3.1.)

[8] Apligraf is a registered trademark of Novartis Pharmaceuticals.

Table 3.1
Worldwide Distribution of Competitive Progenitor Cell Research

Reference	Country	Finding
Gritti et al. (1999)	Italy	Determination of growth factors allowing proliferation of a stem cell-like neural progenitor from adult mouse forebrain.
Bjornson et al. (1999)	Italy/Canada	Adult neural stem cells adopt a hematopoietic fate when transplanted *in vivo*: brain to blood.
Carpenter et al. (1999)	USA	Propagation of long-term, neural stem cells from human fetal forebrain.
Johansson et al. (1999)	Sweden	Identification of neural stem cells in adult mammalian central nervous system.
Uchida et al. (2000)	USA	Development of markers enabling direct isolation of neural stem cell population from human fetal forebrain.
Brooker et al. (2000)	Australia	Insulin-like growth factor-1 promotes specific neural phenotype of propagated neural stem cells from adult mouse forebrain.
Kondo and Raff (2000)	UK	Oligodendrocyte precursor cells from neonatal rats are capable of reverting to multipotent neural progenitor cells.
Clarke et al. (2000)	Sweden	Neural stem cells from adult mouse brain can give rise to cells of all three germ layers when combined with developing embryos *in vivo* indicating a very broad developmental capacity.

It is evident from this very limited example that stem cell research is developing rapidly and is widespread. For this reason, the United States not only does not hold a lead in this area but also must work to remain competitive. Some groups, as would be expected, are indeed competitive as they race to apply their knowledge to a clinical product. Patents licensed to U.S. companies may limit commercial development to the United States for a time, but there is no assurance of long-term dominance in these areas. Patents related to the use of neural stem cells, for instance, have been both competitive and collaborative between groups in the United States and Canada (Table 3.2). A search of new patents and published foreign filings shows this to be a very active area. The availability of funds, infrastructure, and experience for commercial enterprise is still more prevalent in the United States, as evidenced by the fact that of nearly 60 companies presenting at a recent equity research conference, only a handful originated outside the United States (Techvest, LLC's Second Annual Conference on Tissue Repair, Replacement and Regeneration, November 8-9, 2000, New York, NY). However, this infrastructure is developing both in Europe and Japan, where there are several government as well as private initiatives.

The availability of embryonic tissue may play an enabling role in places like the United Kingdom, which recently relaxed some of its restrictions on the use of embryos from *in vitro* fertilization clinics for research. However, evidence in the past year for the existence of pluripotent stem cells in the adult could obviate the need to return to the embryo. Therefore the need for and practical use of ES-derived cell sources for tissue engineering may diminish in the future with a shift in focus to progenitor cells derived from the host or human donor.

Prior to the burst of stem cell activity, there would have been surprisingly little to say regarding progress in living cell therapy or knowledge of the conditions that would enable the practical use of cells in tissue engineering beyond skin. The United States has maintained a lead in the traditional aspects of cell therapy design such as methods of cell encapsulation, design and implementation of extracorporeal liver assist devices, regulation and implementation of autologous cell therapy, and use of allogeneic cells and engineered tissues. Until very recently, identification of culture conditions to effectively cultivate and propagate traditionally hard-to-grow cells types such as the islet cell and the hepatocyte has been rudimentary. This is now changing; thanks to the "stem" cell, *in vitro* culture conditions and *in vitro* environments are now acknowledged as important aspects of interest.

Table 3.2
Rapid Development of Intellectual Property on Neural Stem Cells

Title	Inventors	Patent Assignee	Status
Methods of isolation, enrichment and selection of neural cells and neurosphere initiating cells which are used for treating disorders of the central nervous system.	Buck, D.W. Uchida, N. Weissman, I. (U.S.)	Stem Cells, Inc. (U.S.)	International PCT Application Publication No. WO 00/47762
New neural stem cell cultures—useful in the treatment of conditions, such as epilepsy, stroke, Huntington's disease, Alzheimer's disease, multiple sclerosis, or neuropathies.	Carpenter, M. (U.S.)	Cytotherapeutics, Inc. (acquired by Stem Cells, Inc.) (U.S.)	U.S. Patent No. 5,968,829 U.S. Patent No. 6,103,530
Generating hematopoietic cells from multipotent neural stem cells.	Bjornsen, C.R Reynolds, B.A. Rietze, R.L. Vescovi, A.L. (Canada/Italy)	Neurospheres Holdings, LTD (Canada)	International PCT Application Publication No. WO 98/50433
Producing neurons from population of neural cells containing at least one multipotent stem cell useful for transplantation to treat neurological diseases.	Sorokan, S.T. Weiss, S. (Canada)	Neurospheres Holdings, LTD	U.S. Patent No. 6,165,783
Preparing precursor cells and differentiated cells from neural stem cells—for use in neurological tissue grafting.	Baetge, E.E. Hammang, J.P. Reynolds, B.A. Weiss, S. (U.S./Canada)	Neurospheres Holdings, LTD (Canada)	International PCT Application Publication No. WO 9410292
Re-myelination of neurons using neural stem cells propagated in vitro—either as precursors cells or differentiated oligo-dendrocytes, for treatment of de-myelinating diseases such as multiple sclerosis.	Hammang, J.P. Reynolds, B.A. Weiss, S. (U.S./Canada)	Neurospheres Holdings, LTD (Canada)	International PCT Application Publication No. WO 9409119

U.S. activity is more aggressive and diverse in approach than that of either Europe or Japan. While U.S. academic research, in part out of necessity, tends to favor the use of autologous cells, commercial sourcing is more varied, utilizing cell sources that are autologous (Genzyme Tissue Repair, cultured epidermal sheet grafts, cultured chondrocytes); allogeneic (Organogenesis, bilayered living skin substitute; Advanced Tissue Sciences, living dermal replacement); xenogeneic (Diacrin, porcine fetal neurons; Circe, porcine hepatocytes) and immortalized (Vitagen, immortalized hepatoma cell line). Cell therapies from all four cell sources are used clinically either as commercial products or in current clinical trials. A review of the corporate summaries from the Techvest LLC conference in November 2000 suggests that U.S. commercial strategies for tissue engineering and cell therapy are likely to continue to be diverse in scope. Since the design strategy will be different depending on the cell source used, the diversity seen in U.S. research and development strengthens the U.S. competitive position by creating greater chance of developing truly innovative clinically and commercially viable strategies for cell and tissue therapy and regeneration.

FUTURE POSITION

The United States should continue its advantage in the commercial sector because of its more aggressive approach to implementation and the experience already gained through its current lead position. However, this will depend in part on economic conditions. Further, as Europe and Japan create the infrastructure to encourage and support entrepreneurial enterprise, they will become more competitive. The mindset in

Europe is increasingly entrepreneurial, and change is also occurring in Japan, albeit at a slower pace. In the last few years, top U.S. researchers with entrepreneurial experience have been recruited to Swiss academic institutions, where funding is competitive with that of the United States and where there is freedom to pursue industrial enterprise while maintaining high-level academic positions.

The funding history in the U.S. academic sector, while stimulating the field of tissue engineering, has heavily skewed activity toward an engineering focus. The challenge is for activity by academic laboratories to become more multidisciplinary, with less emphasis on the bioengineering aspects and more, or at least equal, emphasis on the biological aspects of the field. This situation is partially true in Japan as well, which more closely mirrors what is done in the United States. This is not true of Europe, where tissue engineering, while still relatively new, is biologically based. If stem cell biology and related cell culture technology and bioprocessing are to play critical enabling roles in the future, then the United States will be at a distinct disadvantage if it cannot effectively attract and integrate cell and developmental biologists into tissue engineering work.

R&D ACTIVITIES IN EUROPE

Background

European activity in cellular tissue engineering is at a relatively early stage compared to that of the United States. Much of the current strategy for cell therapy revolves around the use of autologous cells. Unlike the United States, little work is being done in Europe in the use of allogeneic cell therapy, despite the fact that two of the largest U.S. commercial enterprises in tissue engineering use allogeneic cells (Advanced Tissue Sciences, Organogenesis). The majority of clinical therapy appears to repeat U.S. work in such areas as epithelial grafts, endothelialization of vascular prostheses, and use of mesenchymal stem cells for bone repair.

Current Condition

Factors determining cell source and design strategy

Although not yet as entrepreneurial as the United States, Europe has a number of initiatives such as the biotechnology incubator facility at the University of Manchester, that are seen as enabling. Manchester is also the recipient of a large government grant for tissue engineering, to be shared with the University of Liverpool Department of Bioengineering. This is an example of an important, deliberate collaboration between a strong matrix biology group and a strong bioengineering group. In Germany, there are government initiatives and funding for startup companies.

The use of autologous cells is seen as a rapid route to clinical use and a product (e.g., Modex, Switzerland), since the use of autologous cells is not yet under regulation. There also appears to be a number of small private laboratories either in clinical trials or with near-term plans for the clinical use of autologous cells. This is in sharp contrast to the situation with respect to allogeneic cells, which in at least one instance, will be regulated as a medicinal product, with all the rigorous requirements that implies. This sharp difference in regulation may serve to keep cell therapy endeavors in Europe at the bench scale, where only hospital institutional review is required. Another factor that will favor the use of autologous cells is the establishment of a cell culture facility in Nantes, France, to facilitate the safe and effective processing of autologous cells for transplant (Bercegeay et al. 1999). The impact of vastly different regulation depending on the cell source may limit development of products with greater scope and market potential until European regulators gain more experience with these types of products and clear development paths and requirements are forged. A more complete discussion on regulatory implications can be found in Chapter 8.

As mentioned above, progress in stem cells is global, with Europe and the United States on near equal footing. Research groups in Milan, Italy, are particularly strong in the area of stem cell research (Istituto di Ricerche Farmacologiche Mario Negri, National Neurological Institute C. Besta). Research based in Genoa, Italy (Centro di Biotecnologie Avanzate), is active in the use of mesenchymal stem cells for bone repair. The UK has taken an active interest in further research on the use of ES cells. This is aided by recent changes in

English law allowing use of human embryos for research. The Imperial College Consortium on Tissue Engineering, funded by the Medical Research Council, has identified ES cell research as one of its focus areas. Although most of its research is in initial phases, this consortium is an important multidisciplinary group with equal biological, clinical, and engineering emphasis.

Europe does not follow U.S. paradigms in tissue-engineering R&D. European researchers appear little aware of or concerned about the U.S. position in the field. There appear to be a number of strategies targeted for local or at least European use, even though the United States constitutes one of the largest markets in the world for these products. This may be due to the fact that tissue engineering is still at a very early stage in Europe, despite the recent increase in activity. This is expected to change as researchers from the United States are recruited abroad and young Europeans, trained in U.S. laboratories, return home. There is also a trend beginning where small European start-up companies set up some portion of their operation in the United States (e.g., Modex, Switzerland; Intercytex, UK.)

JAPAN

Japan is continuing its long history of taking the best of U.S. technology and improving on it (Takeda et al. 1999). For example, researchers at Tokyo Women's Medical University have developed a tissue culture substrate, which modulates cell adhesion properties through changes in temperature, allowing release of epidermal cell sheet from the plate without enzymatic digestion (Takezawa, Mori, and Yoshizato 1990). They intend to use this technology to generate autologous epidermal sheet grafts for patients. The technology of epidermal sheet grafting was developed in the United States over 15 years ago (Rheinwald and Green 1975; Gallico et al. 1984).

Japanese scientists recognize U.S. leadership in tissue engineering and appear keenly aware of U.S. activities in the field. They endeavor to effectively compete with the United States to provide tissue engineering therapies, primarily to their own country. To date, there is still relatively little cell or tissue therapy in Japan. There is little or no xenogeneic therapy at the present time. Like Europe, Japan is currently focused on autologous cell therapy. Although much of what goes on in Japanese laboratories parallels present and previous efforts in the United States, their research appears to be broader in scope, with a stronger biological component than what is currently seen in the United States. This is changing in the United States, but it appears that Europe and Japan have already taken steps to better incorporate biology into their tissue engineering efforts. While European efforts in tissue engineering are emerging, the Japanese have been active participants in tissue engineering for several years. The technology, knowledge and skill base is therefore closer to that of the United States than what is seen in Europe. Japan, however, is not yet competitive in the definition of cellular markers, regulation of cell proliferation, stem cell technology, and other issues of bioprocessing, focusing more on cell interactions with biomaterials (Ohgushi et al. 1999; Nordstrom et al. 1999).

Clinical trial activity exists most notably in the area of bone repair (NAIR, University of Tokyo). In addition, there has been substantial research over the last decade on the development of a liver assist device (e.g., Taguchi et al. 1996; Takabatake, Koide, and Tsuji 1991; Takeshita et al. 1995; Takezawa et al. 2000; Enosawa et al. 2000; Ijima et al. 2000). Although major papers on stem cell biology have not yet appeared from Japanese research groups, this could change quickly. It is believed by some that its lack of restrictions on the use of human fetal cells will enable Japan to develop a leading role in this area. This remains to be seen: given the stiff world competition, Japanese activity in this area must develop rapidly to be competitive. In addition, the rapid development of knowledge and skill surrounding the use of adult-derived stem cells further diminishes Japan's perceived advantage. The paucity of organ donation in Japan due to cultural restrictions that may pose a greater barrier to progress, as it will limit access to adult human cells for progenitor cell research and development.

The Japanese R&D strategy with respect to tissue engineering is quite centralized, with significant government involvement and funding. There is a general strategy to begin with the development of autologous cell therapies and move to allogeneic therapies in the future. There does not appear to be a cultural barrier to the acceptance of tissues made with allogeneic cells, although the sourcing of allogeneic tissues may be problematic for Japan due to the cultural issues. To enable the rapid adoption of autologous

cell therapy, a Cell Science Center is being established in Osaka, which, like the Nantes facility, will provide a central source for safe autologous cell processing. This center is expected to support clinical, industrial, and academic use. The Japanese government is aware of how the entrepreneurial advantage has made a difference in U.S. progress. To this end, there is increasing government support for entrepreneurial enterprise, although some cultural barriers still exist.

SUMMARY

The United States appears to currently retain the lead in the use of cells in tissue engineering. This is in part due to

- availability of tissues through organ donation
- existence of a private sector willing to engage in and invest in diverse approaches to cellular therapy
- existence of three widely available living cell therapies, establishing a regulatory path and providing U.S. regulatory bodies important experience in this area
- a robust academic research history in cell and developmental biology leading to increased potential for breakthrough technologies revolving around stem cells
- a strong academic and industrial presence

U.S. vulnerability in the next several years could come from the following sources:

- inability to attract top biologists to work on tissue engineering problems
- inability to develop strong multidisciplinary teams fast enough to retain a competitive advantage
- fickle private sector support forcing potential technologies to languish or be driven into less effective product development strategies
- widespread growth of stem and progenitor cell research outside of the United States
- insufficient work on basic biological science related to tissue-engineering problems

REFERENCES

Badylak, S., S. Arnoczky, P. Plouhar, R. Haut, V. Mendenhall, R. Clarke, and C. Horvath. 1999. Naturally occurring extracellular matrix as a scaffold for musculoskeletal repair. *Clinical Orthopaedics* (367 Suppl):S333-343.

Badylak, S., B. Kropp, T. McPherson, H. Liang, and P. Snyder, 1998. Small intestinal submucosa: a rapidly resorbed bioscaffold for augmentation cystoplasty in a dog model. *Tissue Engineering* 4 (4):379-387.

Bercegeay, S., A. Cassidanius, C. Darmon, F. Dehaut, P. Lemarre, M.C. Pandolfino, and B. Dreno. 1999. Conception and organization of a cell therapy unit of Nantes, France. *Hematology Cell Therapy* 41 (5):223-228.

Bilbo, P.R., C.J.M. Nolte, M.A. Oleson, V.S. Mason, and N.L. Parenteau. 1993. Skin in complex culture: the transition from "culture" phenotype to organotypic phenotype. *Journal of Toxicology-Cutaneous and Ocular Toxicology* 12 (2):183-196.

Bjornson, C.R., R.L. Rietze, B.A. Reynolds, M.C. Magli, and A.L. Vescovi. 1999. Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. *Science* 283 (5401):534-537.

Block, G.D., J. Locker, W.C. Bowen, B.E. Petersen, S. Katyal, S.C. Strom, T. Riley, T.A. Howard, and G.K. Michalopoulos. 1996. Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGF alpha in a chemically defined (HGM) medium. *Journal of Cell Biology* 132 (6):1133-1149.

Bornemann, R., M.D. Smith, and J.C. Gerlach. 1996. Consideration of potential immunological problems in the application of xenogenic hybrid liver support. *International Journal of Artificial Organs* 19 (11):655-663.

Brannen, C.L., and K. Sugaya. 2000. In vitro differentiation of multipotent human neural progenitors in serum-free medium. *Neuroreport* 11 (5):1123-1128.

Brill, S., P. Holst, S. Sigal, I. Zvibel, A. Fiorino, A. Ochs, U. Somasundaran, and L.M. Reid. 1993. Hepatic progenitor populations in embryonic, neonatal, and adult liver. *Proceedings of the Society for Experimental Biology and Medicine* 204 (3):261-269.

Briscoe, D.M., V.R. Dharnidharka, C. Isaacs, G. Downing, S. Prosky, P. Shaw, N.L. Parenteau, and J. Hardin-Young. 1999. The allogeneic response to cultured human skin equivalent in the hu-PBL-SCID mouse model of skin rejection. *Transplantation* 67 (12):1590-1599.

Brooker, G.J., M. Kalloniatis, V.C. Russo, M. Murphy, G.A. Werther, and P.R. Bartlett. 2000. Endogenous IGF-1 regulates the neuronal differentiation of adult stem cells. *Journal of Neuroscience Research* 59 (3):332-341.

Carpenter, M.K., X. Cui, Z.Y. Hu, J. Jackson, S. Sherman, A. Seiger, and L.U. Wahlberg. 1999. In vitro expansion of a multipotent population of human neural progenitor cells. *Experimental Neurology* 158 (2):265-278.

Catapano, G., M.C. Di Lorenzo, C. Della Volpe, L. De Bartolo, and C. Migliaresi. 1996. Polymeric membranes for hybrid liver support devices: the effect of membrane surface wettability on hepatocyte viability and functions. *Journal of Biomaterial Science Polymers* 7 (11):1017-1027.

Cavallaro, J.F., P.D. Kemp, and K.H. Kraus. 1994. Collagen fabrics as biomaterials. *Biotechnology and Bioengineering* 43: 781-791.

Cheung, A.T., B. Dayanandan, J.T. Lewis, G.S. Korbutt, R.V. Rajotte, M. Bryer-Ash, M.O. Boylan, M.M. Wolfe, and T.J. Kieffer. 2000. Glucose-dependent insulin release from genetically engineered K cells. *Science* 290 (5498):1959-1962.

Cima, L.G., J.P. Vacanti, C. Vacanti, D. Ingber, D. Mooney, and R. Langer. 1991. Tissue engineering by cell transplantation using degradable polymer substrates. *Journal of Biomechanical Engineering* 113: 143-151.

Clarke, D.L., C.B. Johansson, J. Wilbertz, B. Veress, E. Nilsson, H. Karlstrom, U. Lendahl, and J. Frisen. 2000. Generalized potential of adult neural stem cells. *Science* 288 (5471):1660-1663.

Durham, M.M., A.W. Bingaman, A.B. Adams, J. Ha, S.Y. Waitze, T.C. Pearson, and C.P. Larsen. 2000. Cutting edge: administration of anti-CD40 ligand and donor bone marrow leads to hemopoietic chimerism and donor-specific tolerance without cytoreductive conditioning. *Journal of Immunology* 165 (1):1-4.

Ellis, A.J., R.D. Hughes, J.A. Wendon, J. Dunne, P.G. Langley, J.H. Kelly, G.T. Gislason, N.L. Sussman, and R. Williams. 1996. Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure. *Hepatology* 24 (6):1446-1451.

Enosawa, S., T. Miyashita, S. Suzuki, X.K. Li, M. Tsunoda, H. Amemiya, M. Yamanaka, S. Hiramatsu, N. Tanimura, T. Omasa, K. Suga, and T. Matsumura. 2000. Long-term culture of glutamine synthetase-transfected HepG2 cells in circulatory flow bioreactor for development of a bioartificial liver [in process]. *Cell Transplantation* 9 (5):711-715.

Falanga, V., D. Margolis, O. Alvarez, M. Auletta, F. Maggiacomo, M. Altman, J. Jensen, M. Sabolinski, and J. Hardin-Young. 1998. Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. *Archives of Dermatology* 134 (3):293-300.

Fuchs, E., and J.A. Segre. 2000. Stem cells: a new lease on life [in process]. *Cell* 100 (1):143-155.

Gallico, G.G.D., N.E. O'Connor, C.C. Compton, O. Kehinde, and H. Green. 1984. Permanent coverage of large burn wounds with autologous cultured human epithelium. *New England Journal of Medicine* 311 (7):448-451.

Gerlach, J.C. 1996. Development of a hybrid liver support system: a review. *International Journal of Artificial Organs* 19 (11):645-654.

Gritti, A., P. Frolichsthal-Schoeller, R. Galli, E.A. Parati, L. Cova, S.F. Pagano, C.R. Bjornson, and A.L. Vescovi. 1999. Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. *Journal of Neuroscience* 19 (9):3287-3297.

Haynesworth, S.E., M.A. Baber, and A.I. Caplan. 1992. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone* 13 (1):69-80.

Heimbach, D., A. Luterman, J. Burke, A. Cram, D. Herndon, J. Hunt, M. Jordan, W. McManus, L. Solem, G. Warden, et al. 1988. Artificial dermis for major burns. A multi-center randomized clinical trial. *Annals of Surgery* 208 (3):313-320.

Huynh, T., G. Abraham, J. Murray, K. Brockbank, H.O. Hagen, and S. Sullivan. 1999. Remodeling of an acellular collagen graft into a physiologically responsive neovessel. *Nature Biotechnology* 17:1083-1086.

Ijima, H., K. Nakazawa, S. Koyama, M. Kaneko, T. Matsuchita, T. Gion, K. Shirabe, M. Shimada, K. Takenaka, K. Sugimachi, and K. Funatusu. 2000. Conditions required for a hybrid artificial liver support system using a

PUF/hepatocyte-spheroid packed-bed module and its use in dogs with liver failure. *International Journal of Artificial Organs* 23 (7):446-453.

Johansson, C.B., S. Momma, D.L. Clarke, M. Risling, U. Lendahl, and J. Frisen. 1999. Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* 96 (1):25-34.

Kato, Y.P., M.G. Dunn, J.P. Zawadsky, A.J. Tria, and F.H. Silver. 1991. Regeneration of Achilles tendon with a collagen tendon prosthesis. Results of a one-year implantation study. *Journal of Bone Joint Surgery [Am]* 73 (4):561-574.

Koller, M.R., and B.O. Palsson. 1993. Tissue engineering: reconstitution of human hematopoiesis ex vivo. *Biotechnology and Bioengineering* 42:909-930.

Kondo, T., and M. Raff. 2000. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* 289 (5485):1754-1757.

Kropp, B.P., M.K. Rippy, S.F. Badylak, M.C. Adams, M.A. Keating, R.C. Rink, and K.B. Thor. 1996. Regenerative urinary bladder augmentation using small intestinal submucosa: Urodynamic and histopathologic assessment in long-term canine bladder augmentations. *Journal of Urology* 155 (6):2098-2104.

Kubota, H., and L.M. Reid. 2000. Clonogenic hepatoblasts, common precursors for hepatocytic and biliary lineages, are lacking classical major histocompatibility complex class I antigen. *Proceedings of the National Academy of Science USA* 97 (22):12132-12137.

Lagasse, E., H. Connors, M. Al-Dhalimy, M. Reitsma, M. Dohse, L. Osborne, X. Wang, M. Finegold, I.L. Weissman, and M., Grompe. 2000. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo [in process]. *Nature Medicine* 6 (11):1229-1234.

Laning, J.C., J.E. DeLuca, and J. Hardin-Young. 1999. Effects of immunoregulatory cytokines on the immunogenic potential of the cellular components of a bilayered living skin equivalent. *Tissue Engineering* 5 (2):171-181.

Laning, J.C., J.E. DeLuca, C.M. Isaacs, and J. Hardin-Young. 2001. In vitro analysis of CD40-CD154 and CD28-CD80/86 interactions in the primary T cell response to allogeneic "non-professional" antigen presenting cells. *Transplantation* (in press).

Lantz, G.C., S.F. Badylak, M.C. Hiles, A.C. Coffey, L.A. Geddes, K. Kokini, G.E. Sandusky, and R.J. Morff. 1993. Small intestinal submucosa as a vascular graft: A review. *Journal of Investigative Surgery* 6 (3):297-310.

Larsen, C.P., E.T. Elwood, D.Z. Alexander, S.C. Ritchie, R. Hendrix, C.,Tucker-Burden, H.R. Cho, A. Aruffo, D. Hollenbaugh, P.S. Linsley, K.J. Winn, and T.C. Pearson. 1996. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* 381 (6581):434-438.

Lee, R.S., K. Yamada, K.L. Womer, E.P. Pillsbury, K.S. Allison, A.E. Marolewski, D. Geng, A.D. Thall, J.S. Arn, D.H. Sachs, M.H. Sayegh, and J.C. Madsen. 2000. Blockade of CD28-B7, but not CD40-CD154, prevents costimulation of allogeneic porcine and xenogeneic human anti-porcine T cell responses [in process]. *Journal of Immunology* 164 (6):3434-3444.

Livesey, S.A., D.N. Herndon, M.A. Hollyoak, Y.H. Atkinson, and A. Nag. 1995. Transplanted acellular allograft dermal matrix. Potential as a template for the reconstruction of viable dermis. *Transplantation* 60 (1):1-9.

McKenzie, I.F.C., and M.S. Sandrin. 2001. Recent advances in pig-to-primate and related xenotransplantation: A brief review of presentations relating to xenotransplantation at the 18th International Congress of the Transplantation Society, Rome, August 2000. *Graft* 4 (1):83-86.

Newgard, C.B., C. Quaade, A. Thigpen, H.E. Hohmeier, V.V. Tran, F. Kruse, H.-P. Han, G. Schuppin, and S.. Clark. 1999. Engineering of cell lines for diabetes therapy. In Y. Idaka and Y. Yamaoka (Eds). *Tissue Engineering for Therapeutic Use* Vol. 3, pp. 133-139.> Amsterdam, Netherlands: Elsevier Sciences.,

Nordstrom, E., H. Ohgushi, T. Yoshikawa, A.T. Yokobori, and T. Yokobori. 1999. Osteogenic differentiation of cultured marrow stromal stem cells on surface of microporous hydroxyapatite based mica composite and macroporous synthetic hydroxyapatite. *Biomedical Material Engineering* 9 (1):21-26.

O'Connell, P.J., A. Cunningham, and A.J.F. d'Apice. 2000. Xenotransplantation: Its problems and potential as a clinical procedure. *Transplantation Reviews* 14 (1):18-40.

Ohgushi, H., T. Yoshikawa, H. Nakajima, S. Tamai, Y. Dohi, and K. Okunaga. 1999. $A_{l2}O_3$ doped apatite-wollastonite containing glass ceramic provokes osteogenic differentiation of marrow stromal stem cells. *Journal of Biomedical Material Research* 44 (4):381-388.

Oshima, H., A. Rochat, C. Kedzia, K. Kobayashi, and YK. Barrandon. 2001. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* 104 (2):233-245.

Parenteau, N.L. 2000. Cell Differentiation, Animal. In R.E. Spier (Ed.) *Encyclopedia of Cell Technology*, Vol. 1, pp. 365-377. New York : John Wiley and Sons, .

Parenteau, N.L., P. Bilbo, C.J. Nolte, V.S. Mason, and M. Rosenberg. 1992. The organotypic culture of human skin keratinocytes and fibroblasts to achieve form and function. *Cytotechnology* 9 (1-3):163-171.

Pearson, T.C., D.Z. Alexander, M. Corbascio, R. Hendrix, S.C. Ritchie, P.S. Linsley, D. Faherty, and C.P. Larsen. 1997. Analysis of the B7 costimulatory pathway in allograft rejection. *Transplantation* 63 (10):1463-1469.

Petersen, B.E., W.C. Bowen, K.D. Patrene, W.M. Mars, A.K. Sullivan, N. Murase, S.S. Boggs, J.S. Greenberger, and J.P. Goff. 1999. Bone marrow as a potential source of hepatic oval cells. *Science* 284 (5417):1168-1170.

Pittenger, M.F., A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, and D.R. Marshak. 1999. Multilineage potential of adult human mesenchymal stem cells. *Science* 284 (5411):143-147.

Platt, J.L. 1998. New directions for organ transplantation. *Nature* 392 (6679 Suppl):11-17.

Ratner, M.L. 2000. The bricks and mortar of personalized medicine. *Start-Up* December:35-41.

Rheinwald, J.G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell* 6 (3):331-343.

Sanberg, P.R., C.V. Borlongan, A.I. Othberg, S. Saporta, T.B. Freeman, and D.F. Cameron. 1997. Testis-derived sertoli cells have a trophic effect on dopamine neurons and alleviate hemiparkinsonism in rats. *Nature Medicine* 3 (10):1129-1132.

Schuldiner, M., O. Yanuka, J. Itskovitz-Eldor, D.A. Melton, and N. Benvenisty. 2000. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. *Proceedings of the National Academy of Science USA* 97 (21):11307-11312.

Shamblott, M.J., J. Axelman, J.W. Littlefield, P.D. Blumenthal, G.R. Huggins, Y. Cui, L. Cheng, and J.D. Gearhart. 2001. Human embryonic germ cell derivatives express a broad range of developmentally distinct markers and proliferate extensively in vitro. *Proceedings of the National Academy of Science USA* 98 (1):113-118.

Taguchi, K., M. Matsushita, M. Takahashi, and J. Uchino. 1996. Development of a bioartificial liver with sandwiched-cultured hepatocytes between two collagen gel layers. *Artificial Organs* 20 (2):178-185.

Takabatake, H., N. Koide, and T. Tsuji. 1991. Encapsulated multicellular spheroids of rat hepatocytes produce albumin and urea in a spouted bed circulating culture system. *Artificial Organs* 15 (6):474-480.

Takeda, A., K. Kadoya, N. Shioya, E. Uchinuma, M. Tsunenaga, S. Amano, T. Nishiyama, and R.E. Burgeson. 1999. Pretreatment of human keratinocyte sheets with laminin 5 improves their grafting efficiency. *Journal of Investigative Dermatology* 113 (1):38-42.

Takeshita, K., H. Ishibashi, M. Suzuki, T. Yamamoto, T. Akaike, and M. Kodama. 1995. High cell-density culture system of hepatocytes entrapped in a three-dimensional hollow fiber module with collagen gel. *Artificial Organs* 19 (2):191-193.

Takezawa, T., M. Inoue, S. Aoki, M. Sekiguchi, K. Wada, H. Anazawa, and N. Hanai. 2000. Concept for organ engineering: A reconstruction method of rat liver for in vitro culture [in process]. *Tissue Engineering* 6 (6):641-650.

Takezawa, T., Y. Mori, and K. Yoshizato. 1990. Cell culture on a thermo-responsive polymer surface. *Biotechnology (N Y)* 8 (9):854-856.

Uchida, N., D.W. Buck, D. He, M.J. Reitsma, M. Masek, T.V. Phan, A.S. Tsukamoto, F.H. Gage, and I.L. Weissman. 2000. Direct isolation of human central nervous system stem cells. *Proceedings of the National Academy of Science USA* 97 (26):14720-14725.

Uludag, H., P. De Vos, and P.A. Tresco. 2000. Technology of mammalian cell encapsulation. *Advanced Drug Delivery Reviews* 42 (1-2):29-64.

Wang, L., J. Sun, L. Li, D. Mears, M. Horvat, and A.G. Sheil. 1998. Comparison of porcine hepatocytes with human hepatoma (C3A) cells for use in a bioartificial liver support system. *Cell Transplantation* 7 (5):459-468.

Weissman, I.L. 2000. Stem cells: Units of development, units of regeneration, and units in evolution. *Cell* 100 (1):157-168.

_____. 2000a. Translating stem and progenitor cell biology to the clinic: Barriers and opportunities. *Science* 287 (5457):1442-1446.

Widner, H. 1999. Review of allo- and xenogeneic neural grafts in neurodegenerative disorders. Neural Tissue Transplantation Team (NETTLU). *Transplantation Proceedings* 31 (1-2):936-938.

Yagi, M., K.A. Ritchie, E. Sitnicka, C. Storey, G.J. Roth, and S. Bartelmez. 1999. Sustained ex vivo expansion of hematopoietic stem cells mediated by thrombopoietin. *Proceedings of the National Academy of Science USA* 96 (14):8126-8131.

Yannas, I.V., E. Lee, D.P. Orgill, E.M. Skrabut, and G.F.. Murphy. 1989. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. *Proceedings of the National Academy of Science USA* 86 (3):933-937.

Young, J.H., J. Teumer, P.D. Kemp, and N.L. Parenteau. 1997. Approaches to transplanting engineered cells and tissues. In R. Lanza, R. Langer, and W. Chick (Eds). *Principles of Tissue Engineering*, pp. 297-307. Austin, Texas: R.G. Landes CompanyZangani, D., K.M. Darcy, S. Shoemaker, I.P., M.M. 1999. Adipocyte-epithelial interactions regulate the in vitro development of normal mammary epithelial cells. *Experimental Cell Research* 247 (2):399-409.

Zieske, J.D., V.S. Mason, M.E. Wasson, S.F. Meunier, C.J. Nolte, N. Fukai, B.R. Olsen, and M.L. Parenteau. 1994. Basement membrane assembly and differentiation of cultured corneal cells: importance of culture environment and endothelial cell interaction. *Experimental Cell Research* 214 (2):621-633.

CHAPTER 4

BIOMOLECULES

Howard P. Greisler

INTRODUCTION

Tissue engineering principles are based on the utilization of three primary components, namely the biomaterial (whether biological or synthetic), the cell, and the biomolecules, which serve to integrate and to functionally regulate the behavior of the first two. The term "biomolecules" is broad and may overlap with biomaterials and with cells; consequently, it is essential to define the term in the context of the current study. In this report, "biomolecules" refers to all biological materials, whether protein or oligonucleotide species, excluding cells and excluding structural proteins when the latter are used as the biomaterials themselves. Even this relatively limited definition includes agents with a large diversity of functions key to either the assembly of or the structural integrity of tissue engineered constructs or to the functional parameters of that construct. Viewing the population of biomolecules as a whole from the perspective of utilization for the engineering of tissues, a classification to be used in the chapter will include growth factors, differentiation factors, angiogenic factors, and bone morphogenic proteins. While each may be provided or induced as either proteins or as genes, gene transfer technology offers a unique set of technical hurdles, potential advantages and limitations, and potential toxicities; therefore, gene transfer will be considered separately.

Several overarching issues are critical to each of the subdivisions of biomolecules. First and most obvious is the selection of the specific factors to be used. Once selected, a factor may optimally be provided either exogenously or by local delivery, its synthesis by cells induced "endogenously" by the choice of biomaterial, by tissue culture conditions, or by application to the constructs of a specific set of hemodynamic and/or biomechanical forces. In the case of exogenous delivery, the factor may be better provided in either protein or DNA form, and in the latter case, by any of a number of vectors enabling gene transfer, each with its own advantages and limitations. Biomolecules delivered exogenously may be applied locally or administered systemically. In the case of local, or endogenous, delivery, techniques critical to tissue engineering may focus on spatially and temporally controlled bioavailability, the control designed by molecular engineering of the biomolecule itself or of the biomaterial scaffold to which it is applied.

Overview of Issues

1. Induced endogenous production vs. exogenous delivery
2. Selection of specific biomolecules
3. Delivery of delivery protein vs. gene
4. Gene delivery — choice of vector
5. Delivery modality — local vs. systemic
6. Local delivery — controlled bioavailability by engineering of biomolecules or of scaffolds

There does not at this time appear to be any single optimal choice within any of these overarching issues appropriate to all applications within tissue engineering. Given the breadth of the tissue-engineering field, it is likely that advances in all these areas will have an impact on the field as a whole.

As tissue engineering itself is a relatively recent discipline, it is perhaps not surprising that much of the current information on specific biomolecules and on specific delivery systems derives predominantly from the related basic science fields of cellular and molecular biology. The more recent collaborative integration of cellular and molecular biology with biomedical and chemical engineering has allowed current knowledge to be harnessed and applied to the engineering of living tissues. It is likely that this integration will enable future advances in the field. It is similarly likely that those groups fostering the closest research and educational collaborations and cross-fertilization will spearhead future achievements in the field.

GENE TRANSFER

The past decade has witnessed great advances in gene transfer technology, derived largely from the promise of gene therapy applications. Although this promise is largely unrealized to date, new developments in vector design and controlled bioavailability and in controlled bioactivity of the transgene are now being actively applied to tissue engineering designs. The basic principles of gene transfer have came largely from molecular biology laboratories, predominately in the United States. The current efforts to utilize these principles for tissue engineering purposes are centered at institutions facilitating collaborative interaction between molecular biology and related tissue engineering disciplines and as such are occurring globally but still concentrated within the United States.

The key unresolved issues determining the applicability of gene transfer technology include selection of specific gene(s), vector design, delivery modality, scaffold design, and toxicity.

A host of viral and non-viral vectors has been developed, each with inherent advantages and limitations. Detailed lengthy discussions of each are readily available in standard textbooks and review articles. The following short descriptions will address key points only.

Plasmid DNA vectors are used for tissue engineering in laboratories worldwide and offer the significant advantage of avoiding the pitfalls of viral vectors. As such they offer a relatively low risk/benefit relationship. However they tend to be relatively inefficient, with low efficiency expression and transfection, and they are vulnerable to nuclease attack (Bonadio et al. 1999). Most efforts using viral vectors have focused on retroviral, adenoviral and adeno-associated viral (AAV) vectors, although efforts are proceeding with lentiviral and alpha viral vectors as well. Retroviruses are expressed only in proliferating cells, both an advantage and a disadvantage depending on the applications desired. They permanently integrate into genomic DNA.

Toxicity issues include the recent report of induced lymphomas in primates (Simons 2000) Adenoviruses are taken up by both dividing and non-dividing cells, but variable expression efficiencies have been reported. They induce a rather aggressive inflammatory response, and gene expression is limited by immune responses. AAV vectors greatly diminish the inflammatory reactions but possess rather small (<4.5 kb) insertion cassettes, are difficult to produce in large quantities, and immune responses may again interfere with gene expression (Simons 2000). Lentiviruses also integrate into the host genome and are characterized by long duration of expression. However, work in this area is relatively young, and long-term safety is unknown. Alpha viruses induce a very short duration of expression, and again, little is known concerning long-term safety issues.

Key to utilization of gene transfer technologies are future developments in cell- and organ-specific transfer, optimization of efficiency of expression, regulation of expression of the transgene, minimization of local inflammatory and systemic immune reactions, and ability to incorporate large transgenes.

Recent promising developments addressing the above key issues include the following. Optimization of plasmid stability and consequent prolongation of temporal bioavailability have been reported by Lauffenburger and Shaffer (1999). Plasmid stability and efficiency were increased by non-covalent

interactions with peptides, lipids, and cationic dendrimers. DNA-cation complexes in the nanometer size range may be taken up by cells by nonspecific endocytosis. Plasmid half-life may be prolonged by controlling plasmid surface properties with polyethylene glycol. Cell specificity may be controllable by addition of cell targeting ligands for receptor-mediated uptake. Efforts are underway in the area of plasmid encapsulation within polymer scaffolds, for example PLGA, to prolong vector bioavailability by protecting the plasmid from extracellular nucleases (Truong-Le, August, and Leong 1998; Bonadio, Goldstein, and Levy 1998).

A promising approach to regulation of both temporal and spatial bioavailability is the concept of the gene activated matrix (GAM). GAMs are gene therapy biologics consisting of plasmid DNA physically entrapped inside a polymer matrix carrier. Plasmid DNA is a high molecular weight polyanion that is incapable of diffusing through the carrier (for example collagen or fibrin), such that the carrier scaffold holds the DNA *in situ* until the target cells arrive at the GAM site. Bonadio et al. (1999) reported that a 1.0 mg DNA dose per GAM-induced transfection of 30-50% of available canine fibroblasts and local expression of at least picogram amounts of the secreted hPTH transgene product 2-3 weeks after bone-defect and GAM-implant surgery. This concept may be extended to viral vector delivery as well. The Bonadio group has developed a system by which an antibody directed against the adenoviral hexon is applied to the collagen Type I derivatized surface, with subsequent application of the DNA containing adenovirus now sequestered within the GAM. The gene then remains available and stable after implantation until the target invading cell reaches the implant.

Engineering novel scaffolds is a promising approach to regulating gene stability and may allow both prolonged and spatially controlled delivery. Recently, Type I collagen has been produced by recombinant techniques, thus eliminating the risks of disease transmission and allowing a degree of controlled bioavailability (Lamberg et al. 1996; Vuorela et al. 1997).

The synthetic PLGA polymer has been used to control DNA vector bioavailability to coincide with cell ingrowth (Shea et al. 1999); this approach has been studied in the context of medical device coatings (Labhasetwar et al. 1998).

Engineering of complex tissue constructs is likely to require use of multiple genes delivered either sequentially or with site-specific patterns. Advances that are promising for multiple gene delivery include the use of GAMs (Fang et al. 1996); printing technologies to precisely localize genes (Fan et al. 2000); use of multiple polymers with different resorption rates; and positioning of microelectromechanical systems within scaffolds (Santini, Cima, and Langer 1999; Fritz et al. 2000).

A critical unresolved issue for gene transfer application in both gene therapy and tissue engineering is regulation of expression of the transgene product. Few applications or biologic processes would be ideally served by a constitutive expression of basal levels of the selected gene.

A large number of molecular biology laboratories have focused on novel approaches to gene regulation. Promising among them is the recent work that focuses on regulation of gene expression by small-molecular-weight, systemically delivered therapeutic agents. In a paper published in Science in 1999 (Ye et al. 1999), James Wilson and colleagues described a system based on expression of two chimeric human-derived proteins, delivered by *in vivo* somatic gene transfer and reconstituted by rapamycin into a transcription factor complex. Two adeno-associated virus vectors were injected into skeletal muscle of immune-competent mice. One vector expressed a transcription factor chimera; the other expressed erythropoietin under the control of a promoter responsive to that transcription factor. Rapamycin administration yielded a 200-fold induction of plasma erythropoietin, a response persistent for six months in immune-competent mice and at least three months in a rhesus monkey. A similar approach was described in 1997 by Magari et al. (1997), shown diagrammatically in Figure 4.1.

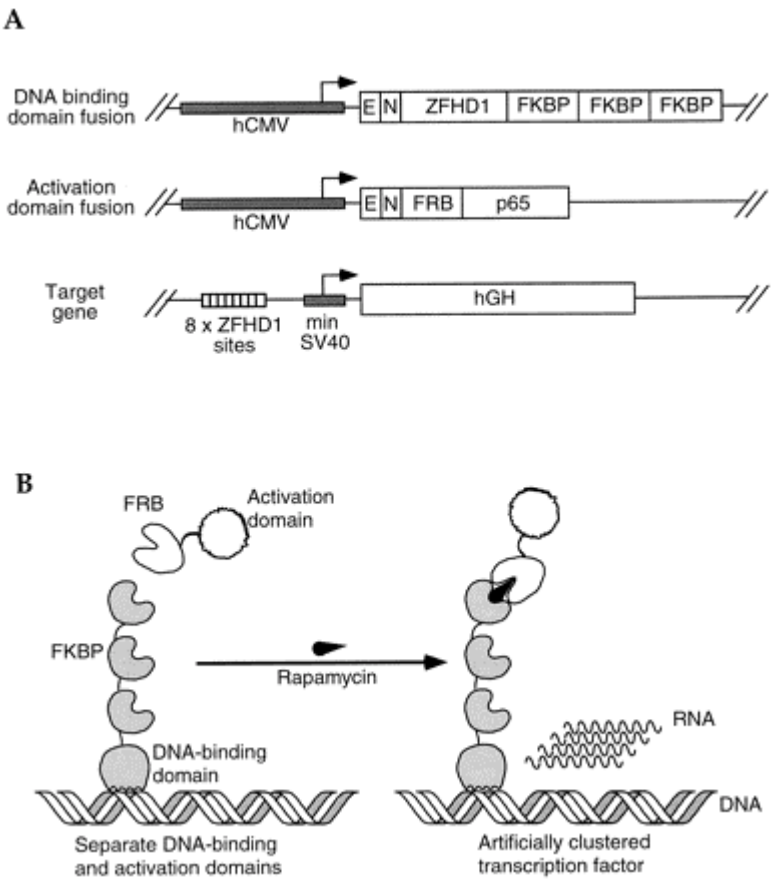


Fig. 4.1. Schematic of the regulated gene therapy system. (A) Schematic diagram of plasmids encoding the reporter gene and transcription factor fusions. The DNA binding domain fusion consists of a DNA binding domain, termed ZFHD1, fused to three copies of hFKBP. The activation domain fusion consists of the transcriptional activation domain from the COOH-terminal region of the NFB p65 protein fused to hFRB. Both fusion proteins are produced under the control of the human cytomegalovirus promoter (hCMV) immediate early promoter and enhancer. An epitope tag (E) and the SV40 T antigen nuclear localization sequence (N) are included at the amino-terminal. The hGH reporter gene consists of a minimal SV40 promoter (min SV40) and eight tandemly reiterated ZFHD1 binding sites. (B) Schematic for rapamycin-dependent protein production. The association of the activation and DNA binding domain fusions occurs only in the presence of rapamycin, which, through different portions of the molecule, binds to both hFKBP and hFRB. Rapamycin-mediated association of the domains results in a fully functional transcription factor that binds to and activates expression of a target gene containing binding sites for ZFHD1 (Magri et al 1997).

Using this approach with the cells doubly transfected *in vitro* and then injected intramuscularly into nu/nu mice, the activation and the DNA binding domain fusions associate only in the presence of rapamycin. The investigators showed a dose-dependent rise in serum human growth hormone (hGH) concentrations in nu/nu mice following either oral or intravenous administration of rapamycin (Figure 4.2).

Furthermore, a single dose of rapamycin, delivered by either the oral or the intravenous route 30 minutes after the intramuscular administration of the transfected cells, induced elevated serum hGH concentrations for at least 12 days (Figure 4.3).

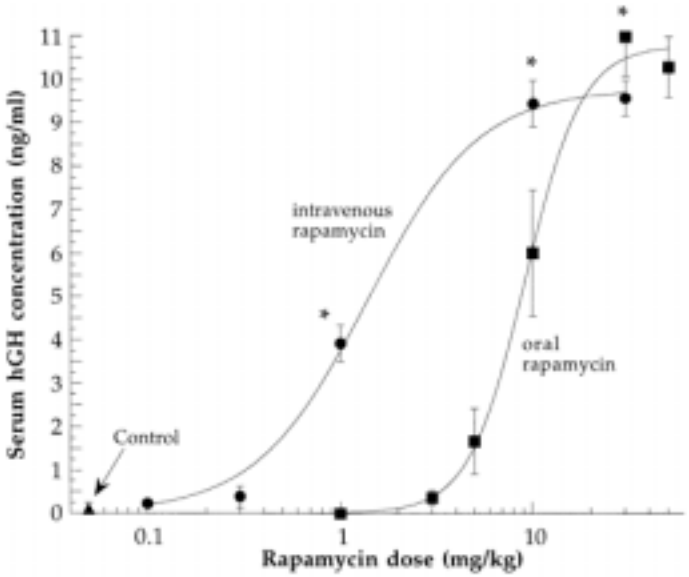


Fig. 4.2. Serum hGH concentration in nu/nu mice receiving HT26-1 cells and various doses of rapamycin. Mice received 2×10^6 HT26-1 cells, a stably transfected clonal cell line derived from HT1080 cells, in four intramuscular sites. Approximately 30 minutes after implantation, the mice received oral doses of rapamycin. The mice were killed 17 hours after rapamycin administration, and blood was collected for hGH determination in serum. Circulating serum hGH concentrations dose-dependently increased in response to rapamycin. Values after intravenous administration of rapamycin are included for comparison (adapted with permission, Assoian and Marcantonio 1996). The ED50 of the oral rapamycin administration was 9.18 ± 0.64 mg/kg, and that of the intravenous administration was 1.38 ± 0.14 mg/kg. Peak hGH levels were independent of the rapamycin administration route. Values are mean ± one SEM, n = at least 5 per point.

*Represents statistical significance from each lower rapamycin dose; $P < 0.05$, one-way ANOVA and Tukey-Kramer multiple comparison testing.

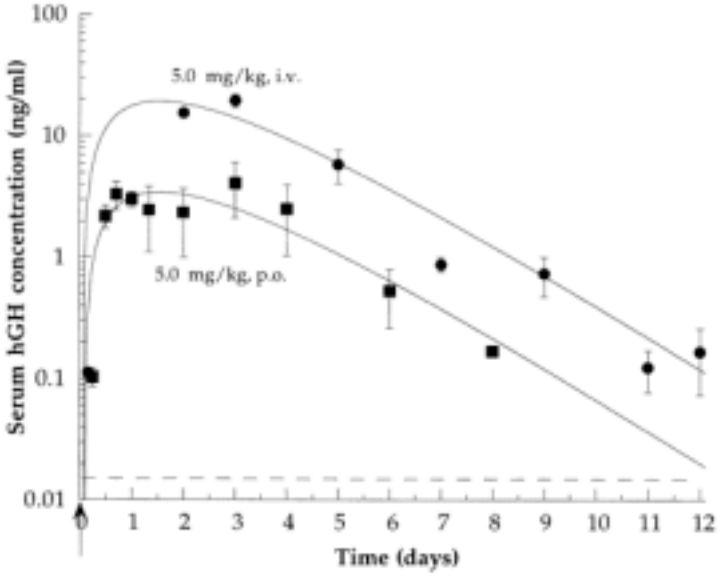


Fig. 4.3. Time course of serum hGH levels after a single rapamycin administration. Mice received 2 x 10^6 HT26-1 cells intramuscularly. Approximately 30 minutes later, they received a 5 mg/kg dose of rapamycin either by the intravenous or by the oral route.

To date this approach has not been evaluated for *in vitro* tissue engineering application but may well be of great value. It allows a method by which the kinetics of expression of the transgene product may be made to coincide with the time of desired effect, thus enabling greater control of the temporal bioavailability of the selected biomolecule(s) used to direct either the assembly of multiple cell-type constructs or the phenotypic characterization or differentiation of the cells so assembled.

Another hurdle to regulation of transgene expression is the frequently short durability of expression. In some cases this may result from the phenomenon of "gene silencing." As described by Timothy Bestor (2000), transcriptional silencing may result from insertion of retroviral DNA or incorporation of repeat arrays of the inserted sequences, triggering methylation of DNA within regulatory regions. Post-transcriptional gene silencing and RNA interference (RNAi) can similarly induce degradation of homologous RNA. These observations led Bestor to state, "Even if the delivery and regulation problems can be solved, it is not unlikely that successful gene transfer and tissue-specific expression may be followed by loss of therapeutic effect unless silencing-resistant expression constructs are developed and used" (Bestor 2000).

It is likely that optimization of vector design and utilization of silencing-resistant expression constructs, along with advances in techniques for regulation of expression of these "better" designed constructs, will impact directly on strategies for using gene transfer techniques in the field of tissue engineering.

The final major limitation of gene transfer application is the critical area of safety and toxicity. Among concerns are those related to persistence and bioavailability of the vectors themselves as well as to regulation of the pharmacokinetics and pharmacodynamics of the vector-encoded proteins. Cytotoxicity may be induced at the local delivery site as, for example, the potential for retrovirus-induced cellular transformation (Friedman 2000). Systemic cytotoxicity remains a major concern as well. Adenoviral vectors have been clearly associated with immune activation. Plasmid vectors with CpG motifs have been reported to activate lymphocytes and induce immune suppression (Krieg 1999).

The gene transfer approach to delivery of biomolecules for tissue engineering applications remains a highly promising strategy for sustained and effective expression of selected biomolecule(s). However, critical development is required to optimize efficiency of transfection and regulation of gene expression, control of temporal and spatial bioavailability, and minimization of cytotoxicity.

Major advances in these areas have stemmed predominately from molecular biology laboratories, most prominently those in the United States, primarily with the goal of gene therapy application. Application of these advances to tissue engineering is likely to be an emerging focus of laboratories internationally, with well-integrated collaboration between molecular biologists and tissue engineers.

ANGIOGENIC FACTORS/GROWTH FACTORS

The topics of angiogenic factors and growth factors are segregated only artificially, inasmuch as angiogenesis necessarily involves cell proliferation, and great overlap exists such that most angiogenic factors are directly or indirectly growth factors as well, and many growth factors have some angiogenic potency. Thus these factors will be discussed together.

Cell viability and function is dependent upon nutrient supply and oxygenation. While diffusion may be sufficient when cells are within 100-200 microns of perfusing blood, larger tissue constructs must be provided with both an infiltrating capillary network and a communication between that capillary network and the host arterial and venous systems. Both may be provided separately as, for example, when utilizing biomolecules to induce infiltration of capillaries followed by surgically established connections to the host circulation. The provision of such a capillary network must be based upon utilization of endothelial cells co-cultured with the other cell types within that tissue construct. Critical issues are cell sourcing of the endothelia, which are generally potent antigen-presenting cells and thus activate immune processes when allogeneic or xenogeneic cells are employed. Consequently, active research programs are focused on use of autogeneic endothelial cells or endothelial progenitor stem cells, or are focused on novel strategies of either immunosuppression or blockade of cellular antigen presentation.

Critical as well is the spatial distribution of the infiltrating capillary network. The former issue is discussed in more detail in Chapter 3 of this report, "Cells," while the latter is covered more completely in Chapter 2, "Biomaterials" and Chapter 6,"Engineering Design Aspects of Tissue Engineering."

Recognized angiogenic factors include members of the FGF family, notably FGF-1, 2, 4, and 5, and members of the VEGF family, A-E. The FGFs tend to be potent yet relatively nonspecific growth factors with some angiogenic activity, while the VEGF group trends to be relatively more specific to angiogenesis but with relative endothelial cell-specific, yet weaker, endothelial cell mitogenicity. Other recognized angiogenic factors include PDGF, AA, AB and BB, HGF (scatter factor), the angiopoietins, HIF-1α, IL-6 and IL-8, TNFα, nitric oxide, PAF, substance P, and tissue factor.

An important series of reports on the mechanism of and the *in vitro* biomolecular induction of angiogenesis has been published by Michael Pepper and colleagues in Geneva (Montesano 1992). Endothelial cells cultured in a monolayer on fibrin or collagen gels may be induced to invade into the depth of the gels and form infiltrating capillary-like tubular structures when either FGF-2 or VEGF is introduced into the gels (Figure 4.4). The distance of capillary infiltration is proportional to the concentration of FGF or VEGF, and an apparent synergism between these angiogenic factors is well described (Figure 4.5).

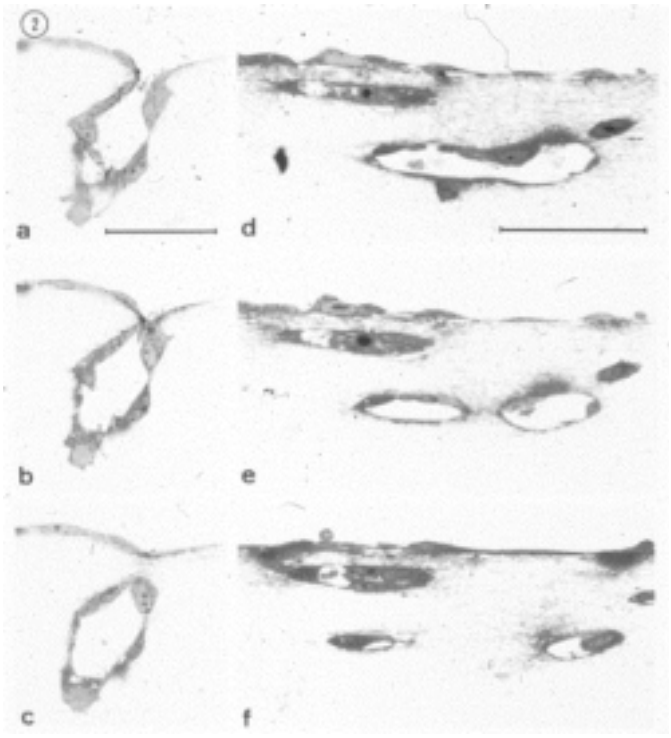
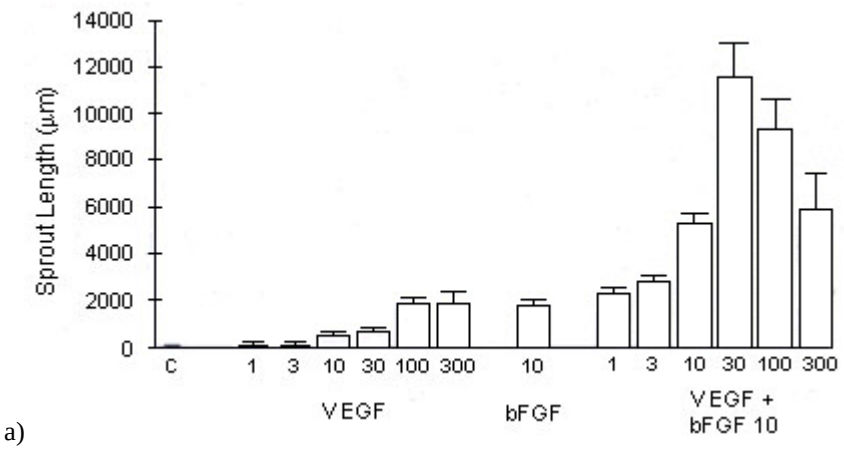
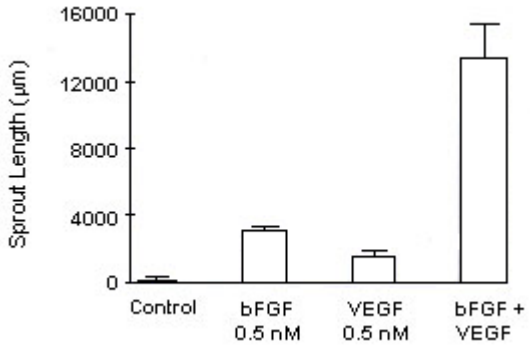


Fig. 4.4. Images a-c show the invasion of collagen gels and formation of vessel-like structures by PMA-treated microvascular endothelial cells. Consecutive serila sections (d-f) show the continuity between the endothelial cells forming the surface monolayer and those delimiting a tube-like structure inside the collagen matrix. The serial sections show the branching of a vessel-like structure into two smaller tubes that progressively diverge from one another. Bar = 50 μm. (Montesano 1992.)

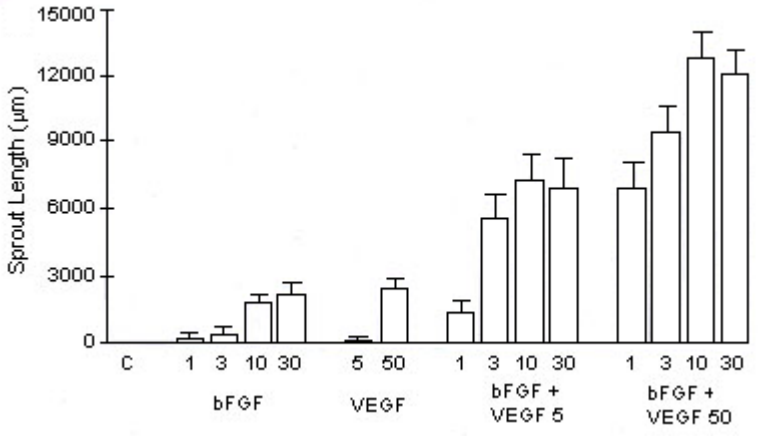
These observations have recently led to *in vivo* application of therapeutic angiogenesis. Efforts have focused on delivery of either FGF or VEGF family members in either protein or DNA form into ischemic tissue with documented induction of capillarization. Whether such a strategy may be sufficient in and of itself for the desired clinical result is an open question but these efforts point to the validity of the approach for inducing angiogenesis within tissue engineered constructs. Similar efforts have focused on use of these factors to induce angiogenic mechanisms by which infiltrating capillaries may provide a source of autologous endothelium to form a monolayer at the blood contacting surface of implanted synthetic or tissue engineered vascular constructs such as blood vessels, hearts, and heart valves.



a)



b)



c)

Fig. 4.5. Quantitation of $VEGF_{165}$ and bFGF-induced *in vitro* angiogenesis. Randomly selected fields of BME cell monolayers treated with $VEGF_{165}$ and/or bFGF for 4 days were photographed at a single level beneath the surface monolayer. Endothelial cell invasion was quantitated by measuring the total length of all cell cords that had penetrated beneath the surface monolayer. (a) $VEGF_{165}$ dose - response and effect of co-addition of bFGF. Values for $VEGF_{165}$ and bFGF on the abscissa are in ng/ml. (b) Comparison of equimolar (0.5 nM) concentrations of $VEGF_{165}$ (22.5 ng/ml) and bFGF (9 ng/ml) and effect of co-addition of VEGF and bFGF. (c) bFGF dose-response and effect of co-addition of $VEGF_{165}$. Values for $VEGF_{165}$ and bFGF on the abscissa are in ng/ml. Results in (a), (b), and (c) are from three photographic fields per experiment of at least three separate experiments, i.e., a total of at least nine photographic fields per condition, and are expressed as mean ± SEM.

The term angiogenesis must be distinguished from arteriogenesis and vasculogenesis. Angiogenesis refers to newly formed capillaries derived *in vivo* from post-capillary venules by endothelial cell migration, proliferation, and matrix degradation. Expression of both angiogenic factors and their cellular receptors is modulated by ischemia, hypoxia, and inflammation. It has been stated that there can be no angiogenesis in the absence of inflammation (Jones et al. 1999). By contrast, arteriogenesis involves formation of muscular arteries containing all three wall layers—intima, media, and adventitia—and is modulated both by inflammatory mediators such as those derived from activated macrophages and by shear stress. Vasculogenesis refers to the development of new vascular structures from pluripotent stem cells occurring in embryogenesis and which may take place in adult tissues under specific physiologic and possibly pathologic conditions.

Application of angiogenic factors with a specific goal of vascularization of tissue-engineered constructs is exemplified by work reported by Aijoka and colleagues (1999) from the Tokyo Institute of Technology. This group used VEGF-transfected hepatocytes transplanted intraperitoneally on collagen beads in mice; they demonstrated dramatically enhanced capillarization. Significant enhancement of hepatocyte growth was noted as well, either due directly to secreted VEGF or more likely due indirectly either to additional factors provided by the endothelium or to the provision of greater perfusion (Figure 4.6).

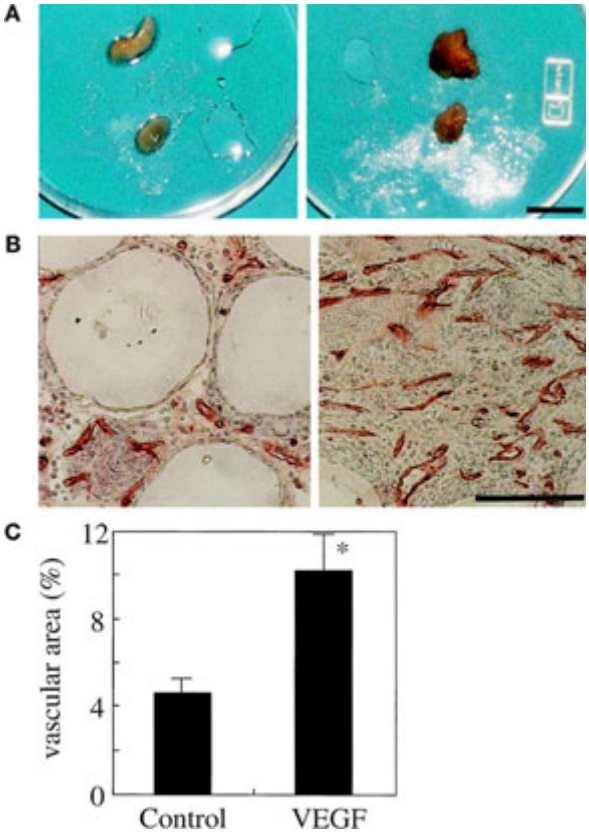


Fig. 4.6. Development of blood vessel network in VEGF-transplanted hepatic tissues. Untreated or VEGF-transfected spheroidal hepatocytes (4.8×10^6) were transplanted, and 2 weeks later established conglomerates were dissected and fixed. (A) Established transplanted hepatic tissues. Note that VEGF-treated transplants are larger in size and the intensity of the red color is higher than that of control tissues. Control bar: 1 cm. (B) Cryostat sections (10 μm) immunostained with anti-CD31 antibody. (*Left*) control tissue; (*right*) VEGF-transfected tissue. Control bar: 100 μm. (C) Five randomly selected fields of tissue sections were analyzed by National Institutes of Health image software, and the area of blood vessels stained with anti-CD31 antibody was estimated. *P < .02. (Aijoka 1999.)

Biomaterial scaffolds differing in either surface or bulk composition or in biomechanical properties may differentially induce cellular ingrowth and may modulate cellular functional characteristics. Greisler and colleagues (1985; 1986; 1987; 1988; 1989; 1991; and 1993) have documented that vascular prostheses

woven from lactide/glycolide copolymeric yarns elicit *in vivo* tissue incorporation dissimilar to that induced by similarly woven polyethylene terephthalate prostheses in animal models. The implanted bioresorbable polymers induce transinterstitial capillary-rich mesenchymal tissue ingrowth, dominated by myofibroblasts, and matching the kinetics of observable macrophage and foreign body giant cell phagoytosis of the polymers. The tissue ingrowth is effected by induced migration and cell proliferation with identical kinetics to prosthetic resorption. *In vitro* analyses showed that the lactide/glyolide family differentially activates macrophages to upregulate synthesis of mitogenic agonists capable of inducing proliferation of endothelial cells, smooth muscle cells, and fibroblasts, 50-80% of the activity immunoreacting with and blocked by neutralizing antibodies to FGF-2 (Greisler et al. 1991; Greisler et al. 1989). Thus, the differential response to these biomaterials is modulated by biomolecules.

In related studies, exogenous FGF-1 has been delivered from fibrin gel suspensions impregnated into vascular prostheses. These implanted constructs induced a significant cellular proliferative response and tissue incorporation with extensive capillarization yielding enhanced endothelialization of blood contacting surfaces (Figures 4.7 and 4.8).

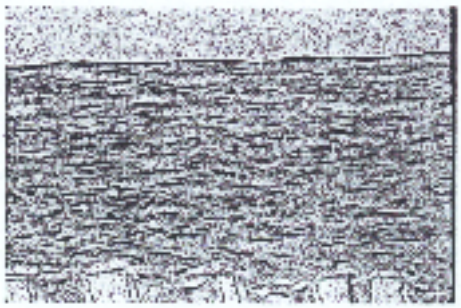


Fig. 4.7. Fibrin Gel/FGF-1/Heparin Treated ePTFE graft canine Thoracoabdominal aortic bypass, 20 weeks.

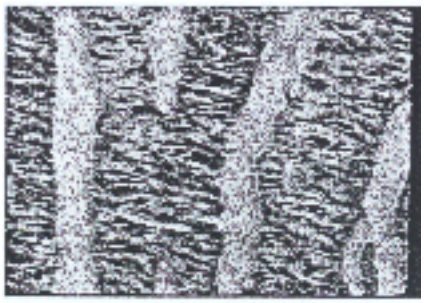


Fig. 4.8. Fibrin Gel/FGF-1/Heparin Treated ePTFE graft canine Thoracoabdominal aortic bypass, 20 weeks.

This *in vivo* response was consistent with the *in vitro* tube formation described above and reported by Pepper et al. (Montesaro 1992).

Most angiogenic factors and growth factors are relatively nonspecific as to the cell type affected. Therefore, an approach for establishing cell specificity and/or altering functional potency is site-directed mutagenesis to alter either ligand/receptor interactions or intracellular processing, or to synthesize chimeric factors to which cell targeting ligands are attached. These approaches have been used by Burgess, Maciag, and Greisler (Lin et. al. 1998; Shireman et al. 1998; Shireman et al. 2000; Xue et al. 2000; and Xue et al. in press). Several recombinantly produced mutations of FGF-1 have been shown to significantly augment the strength of the mitogenic signal when tested on endothelial cells and/or smooth muscle cells, including the replacement of the three cysteine residues by lysine and the replacement of the serine at the 130 position (within the heparin binding domain) with lysine (Xue et al. 2000). This approach may be beneficial for promoting the molecular stability of the protein within delivery vehicles based on fibrin, which contains the proteolytically active enzyme thrombin (Shireman et al. 2000). The addition to the growth factor of an HB-GAM targeting sequence that interacts with cell surface heparan sulfate proteoglycans, including the syndecan family, may

yield a relative cell specificity. The HB-GAM/FGF-1 chimera augments the relative endothelial cell smooth muscle cell mitogenicity from that induced by wild type FGF-1 (Xue et al. in press).

Thus the application of angiogenic and other growth factors within delivery systems impregnated into tissue engineered constructs may promote desired tissue responses. These may be optimized by molecular engineering of the structure of the naturally occurring protein or by synthesis of novel protein structures.

Hubbell and colleagues in Zurich have utilized novel strategies by which to incorporate biologically active molecules into fibrin gels to either selectively promote attachment and/or migration of selected cell types, or to deliver growth factors to cells recruited by the vehicle-containing constructs (Hubbell 1995). Adhesion-promoting oligopeptides based on primary structures of receptor-binding domains of extracellular matrix adhesion proteins such as fibronectin and laminin were shown to display similar receptor specificity and binding affinity as the whole protein (Yamada 1991, Hubbell 1995). Thus RGD, YIGSR, REDV and other sequences may be affixed to biomaterial scaffolds or natural tissues to selectively promote attachment of relatively specific cell types. Interestingly cell attachment and cell migration may both vary in relation to the relative density of the adhesion peptide/receptor interactions and either attachment or migration may be selectively promoted by modulating these interactions.

A novel approach to fibrin-based delivery has been developed by Hubbell (Sakiyama-Elbert and Hubbell 2000; Schense and Hubbell 1999). Fibrin forms naturally by thrombin cleavage of fibrinogen, followed by self-assembly into fibrin monomer, then polymerized in the presence of Factor XIII, which itself is activated in the presence of thrombin. Using a method of covalently cross-linking bi-domain peptides to fibrin matrices, these investigators have placed the Factor XIIIa substrate from α2-plasmin inhibitor at the amino terminus and a heparin-binding domain at the carboxy terminus, thus covalently incorporating the heparin-binding peptide. This strategy has been used to immobilize both heparin-binding peptides and other receptor-binding peptide sequences for recognition by cells recruited into the fibrin gels. Using RGD containing bi-domain peptides cross-linked into fibrin gels at concentrations up to 8.2 mol of peptide/mol of fibrinogen, dorsal root ganglia were cultured within the gels. Both two- and three-dimensional neurite outgrowth demonstrated a bi-phasic dependence on RGD concentration with maximal neurite extension promoted by intermediate adhesion site densities (Figure 4.9) (Sakiyama, Schense, and Hubbell 1999; Schense and Hubbell 1999).

This system may similarly allow for immobilization of biologically active growth factors. When the bi-domain peptide includes a heparin-binding domain, this covalently bound peptide may be used to electrostatically bind applied heparin, which in turn may serve to sequester heparin-binding growth factors. These factors could then be available to recruited cells upon release by cellular heparinase or plasmin (Sakiyama, Schense, and Hubbell 1999; Schense and Hubbell 2000). Synthetic bioresorbable scaffolds for growth factor delivery have been utilized by Merkle and colleagues. Using PLGA microspheres, IGF-1 delivery has shown progress for osteoinduction, and NGF delivery from PLGA microspheres in hydrogels has been used for nerve guidance conduits.

In vivo application of biomolecules has been described using photopolymerized polyethyleneglycol diacrylate precursors. Using this approach, a bilayer hydrogel depot was applied to the luminal aspect of arteries (An et al. 2000). The initial high-permeability layer containing the biomolecules is applied to the arterial surface followed by a second more low-permeability layer to shift the relative diffusion of protein toward the underlying arterial wall.

Gene transfer techniques for growth factor delivery have been reported by Takahama et al. (1999). This group has focused on FGF-4 (HST-1) using adenoviral delivery from atelocollagen pellets implanted or injected into mice. This member of the FGF family has a signal sequence enabling effective secretion of the transgene product from transfected cells. Protein expression has been observed beyond 60 days with data supporting possible applicability of this approach to preserve platelet production during radiation therapy or chemotherapy.

Thus both *in vitro* and *in vivo* cell recruitment to and function within tissue-engineered constructs are mediated either directly by exogenously applied biomolecules or indirectly by biomaterial-induced cellular synthesis and release of these biomolecules. Strategies likely to advance tissue-engineering concepts include use of molecular modifications of the biomolecules themselves or the development of novel delivery vehicles

and scaffolds to coordinate the temporal and spatial distribution of the biomolecules in relation to the desired cellular response. These strategies are currently under intense investigation internationally.

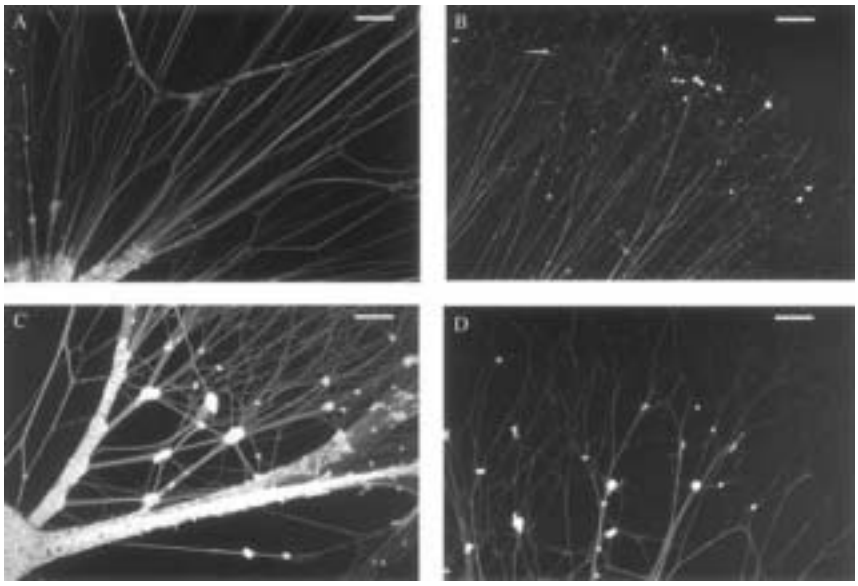


Fig. 4.9. Images of DRGs cultured within fibrin gels with and without heparin binding peptide. (A) Unmodified fibrin near ganglion body. BB) Unmodified fibrin near growth cones. (C) Fibrin containing 2PI1–7-ATIII121–134 heparin-binding peptide near ganglion body. (D) Fibrin containing 2PI1–7-ATIII121–134 peptide near growth cones. Confocal scanning laser microscopy of DRGS was performed using 10x magnification. The images shown are extended focus projections of ~50–100 images taken at 7–10 μm intervals. The scale bar represents 100 μm. Cells were stained with fluorescein diacetate prior to imaging. (Sakiyama 1999.)

DIFFERENTIATION FACTORS

Differentiation of pluripotent and multipotent stem cells and modulation of key phenotypic characteristics of adult cells may be selectively induced by application of biomolecules affecting these processes. Embryologic cellular differentiation is regulated by complex interactions of cytokine and growth factors acting via autocrine, paracrine, and endocrine pathways. The vast potential of stem cell technology for tissue engineering will be greatly impacted by furthering our understanding of the regulation of these differentiation processes. The availability of embryonic and adult stem cells and control of their phenotypic differentiation may significantly resolve immunologic barriers to the use of allogeneic cells. Recent work has similarly shown many adult mesenchymal cells to be capable of a degree of transdifferentiation controlled by cell-matrix and cell-cell interactions mediated by the biomolecular environment.

In the normal muscular artery *in vivo*, vascular smooth muscle cells are highly contractile and display relatively low indices of proliferation or of protein synthesis. Once injured, for example by interventional angioplasty procedures or by commonly used *in vitro* culture techniques, the cells undergo significant phenotypic modulation from the “contractile” to the “synthetic” or “proliferative” phenotype, identifiable both by ultrastructural morphology and by functional parameters. This “de-differentiation” process likely plays a role in the restenosis lesion and similarly must be controlled for purposes of vascular tissue engineering.

Recent studies have pointed to the significant impact of extracellular matrix proteins in modulating smooth muscle cell differentiation. Assoian and colleagues (Assoian and Marcantonio 1996) demonstrated that vascular smooth muscle cells cultured on a fibronectin substrate adopt a proliferative/synthetic phenotype. The fibronectin ligand interacts with the $\alpha 5\beta 1$ integrin receptor and induces ras activation. By contrast, vascular smooth muscle cells cultured on a laminin substrate, which interacts with the $\alpha 3\beta_1$ integrin receptor, do not undergo ras activation, and retain a contractile/nonproliferative phenotype, This latter group lacks tyrosine phosphorylation in focal adhesions and detectable focal adhesion kinase (FAK) activity. Thus *in*

vitro smooth muscle cell activation likely depends in part upon formation of focal adhesions with associated tyrosine kinase activity and cytoskeletal reorganization. Such integrin clustering and cytoskeletal reorganization is followed by mitogen-activated protein kinase (MAPK) activity. Here signal transduction elicited by integrins and by growth factor receptors synergize. Thus, for engineering of the arterial media, rich in smooth muscle cells, a laminin-based substrate may be advantageous.

A similar approach has been utilized by Oshima at the University of Tsukuba for purposes of hepatic tissue engineering. This group has made considerable progress in the use of rat and porcine hepatocytes cultured on porous polyvinyl formyl resins for treatment of patients with acute hepatic failure. Data suggests that hepatocytes when cultured on a laminin substrate show enhanced albumin secretion.

An important development underway in laboratories internationally is the use of specific differentiation factors within defined culture media to selectively promote growth of a single cell type to be used for tissue-engineering applications. Intense study of such defined media is in progress in Japan at RIKEN for selective expression of CTL (cytotoxic T lymphocytes) or NK (natural killer) cells. Similarly at the Virchow Campus of Hybrid Organ GmbH, work is in progress on the development of defined media for selective hematopoietic cell isolation and expansion.

The potential impact of these approaches is great to selectively expand single differentiated cell types and to regulate and induce desired phenotypic characteristics for optimal function of tissue engineered constructs.

BONE MORPHOGENIC PROTEINS

Bone morphogenic proteins represent a family of related osteoinductive peptides akin to differentiation factors. The clinical need in patients with non-healing fractures and osteoarthritis is immense. In addition to the family of BMPs, osteoinduction may be promoted by a number of growth factors. The complexity, however, is such that significant osseous formation is also dependent upon induction of angiogenesis for vascular supply and maintenance of newly formed osteoblasts.

The group at Genetics Institute (Georgia) has focused on the use of rhBMP-2 and has identified its cellular receptors and signaling pathways. Using local administration of rhBMP-2 in collagen-based biomaterial matrices, the group has shown *in vivo* osteoinduction matching the anatomic site of the implant (Morris 2000). Using this system in a human clinical trial, Boden et al. (2000) randomized patients with single-level lumbar degenerative disk disease refractory to nonoperative management. Fourteen patients received lumbar interbody arthrodesis with a tapered cylindrical threaded fusion cage filled with rhBMP-2/collagen sponge (N=11) or autogenous iliac crest bone (n=3). Serial radiographs at 6, 12, and 24 months showed all patients with the rhBMP-2 implants to have progressive ossification and solid fusions, compared to 2 of 3 of the control group (Figure 4.10).

The group at Imperial College has focused on embryonic stem cell osteogenic induction by application of IGF and NO with promising *in vitro* results. Otsuka and colleagues in Osaka (1997) have shown the importance of FGF-2 for regeneration and repair of rabbit full thickness defects in articular cartilage. Administration of FGF-2 induced regeneration of articular cartilage and subchondral bone in lesions too large to repair spontaneously. Undifferentiated mesenshysmal cells infiltrated the lesions and initiated chondrogenic differentiation resulting in the resurfacing of the defects with hyaline cartilage and recovery of the subchondral bone 8 weeks after lesion creation. The chondrogenesis was eliminated in animals treated additionally with a monoclonal antibody against FGF-2 (Figure 4.11 and Table 4.1).

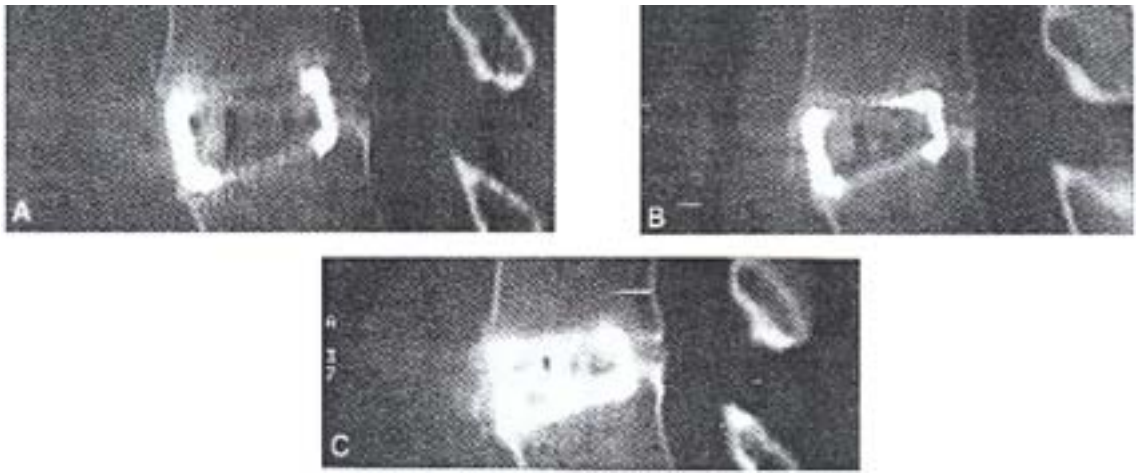


Fig. 4.10. Sagittal reformations of a computed tomography scan from a patient who underwent anterior lumbar interbody arthrodesis with a titanium threaded fusion device filled with rhBMP-2/absorbable collagen sponge instead of autogenous bone graft. (A) At 3 months after surgery bone formation is shown throughout the cage, as well as partial anterior bridging in front of the cage. (B) At 6 months after surgery, bone growth throughout the center of the cage and a complete bridge anterior to the cage are found. (C) At 1 year after surgery, more dense bone filling the entire cage, persistence of the anterior bridge of bone, and formation of a bridge bone posterior to the cage are found.

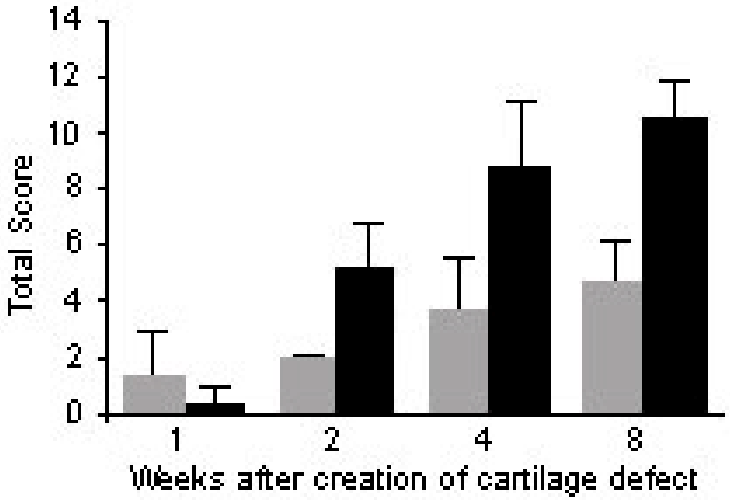


Fig. 4.11. Histological scores for articular cartilage repair of the 5 mm diameter defects treated with saline alone or treated with 50 pg/h of FGF-2. Sections were examined independently by two observers, who allotted scores in accordance with a semi-quantitative histological grading scale (Table 4.1). Values are the means ± SD of the scores made on histological sections from five individual animals. (Pineda et al. 1992.)

Table 4.1
Scoring System for the Histological Appearance of Full-Thickness Defects of Articular Cartilage*

Characteristic	Score†
Filling of defects (%)	
125	3
100	4
75	3
50	2
25	1
0	0
Reconstitution of osteochondral junction	
Yes	2
Almost	1
Not close	0
Matrix staining	
Normal	4
Reduced staining	3
Significant staining	2
Faint staining	1
No staining	0
Cell morphology	
Normal	4
Mostly hyaline and fibrocartilage	3
Mostly fibrocartilage	2
Some fibrocartilage, but mostly non-chondrocytic cells	1
Non-chondrocytic cells only	0
Perfect Score	14

*Modified from Pineda et al. (1992).
† semi-quantitative score of 1-14 with 14 as a perfect score

REFERENCES

Aijoka, I., T. Akaike, and Y. Watanabe. 1999. *Hepatology* 29 (2):396-402.

An, Y., and J. Hubbell. 2000. *J. Controlled Release* 14:64 (1-3), 205-215.

Assoian, R.K., and E.E. Marcantonio. 1996. *J. Clinical Investigation* 98:2436.

Bestor, T. 2000. *J. Clinical Invest.* 105:409-411.

Boden, S., T. Zdeblick, H. Sandhu, and S. Heim. 2000. *Spine* 25 (3):376.

Bonadio, J., S.A. Goldstein, and R.J. Levy. 1998. *Advanced Drug Delivery Reviews* 33:53.

Bonadio, J., E. Smiley, P. Patil, and S. Goldstein. 1999. Localized, direct plasmid gene delivery *in vivo*: Prolonged therapy results in reproducible tissue regeneration. *Nature Medicine* 5 (7):753–759.

Fan H., Y. Lu, A. Stump, S.T. Reed, T. Baer, R. Schunk, V. Perez-Luna, G.P. Lopez, and C.J. Brinker. 2000. *Nature* 405:56.

Fang, J, Y.-Y. Zhu, E. Smiley, J. Bonadio, J.A. Rouleau, S.A. Goldstein, L.K. McCauley, B. Davidson, and B. Roessler. 1996. *Proc. Natl. Acad. Sci. U.S.A.* 93:5753.

Friedman, T. 2000. *Science* 287:2163.

Fritz, J., M.K. Baller, H.P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.-J. Guntherodt, C. Gerber, and J.K. Gimzewski. 2000. *Science* 288:316.

Greisler, H.P. 1991. *J. Vasc. Surg.* 13:748-750.

Greisler, H.P., C.W. Tattersall, J.J. Klosak, E.A. Cabusao, J.D. Garfield, and D.U. Kim. 1991. *Surgery* 110:645-655.

Greisler, H.P., E.B. Cabusao, T.M. Lam, P.M. Murchan, J. Ellinger, and D.U. Kim. 1991b. *American Society for Artificial Internal Organs Transactions* 37:M472-M475.

Greisler, H.P., J.W. Dennis, E.D. Endean, J. Ellinger, R. Friesel, and W.H. Burgess. 1989. *J. Vasc. Surg.* 9:588-593.

Greisler, H.P., J.W. Dennis, E.D. Endean, and D.U. Kim. 1988. *Circulation Supplement I* 78:I6-I12.

Greisler, H.P., E.D. Endean, J.J. Klosak, J. Ellinger, J.E. Dennis, K. Buttle, and D.U. Kim. 1988. *J. Vasc. Surg.* 7:697-705.

Greisler, H.P., S.C. Henderson, and T.M. Lam. 1993. *J Biomaterials Science, Polymer Ed.* 4:415-430.

Greisler, H.P., D.U. Kim, J.W. Dennis, J.J. Klosak, K.A. Widerborg, E.D. Edean, R.M. Raymond, and J. Ellinger J. 1987. *J. Vasc. Surg.* 5:572-583.

Greisler, H.P., D.U. Kim, C. Fenoglio, J.B. Price, and A.B. Vorhees. 1985. *Arch. Surg,* 120:315-323.

Greisler, H.P., D. Petsikas, T.M. Lam, N. Patel, J. Ellinger, E. Cabusao, C.W. Tattersall, and D.U. Kim. 1993. *J. Biomedical Material Research* 27:955-961.

Greisler, H.P., T.H. Schwarcz, J. Ellinger, and D.U. Kim. 1986. *J. Vasc. Surg.* 3:747-756.

Greisler, H.P., C.W. Tattersall, J.J. Klosak, E.A. Cabusao, J.D. Garfield, and D.U. Kim. 1991. *Surgery* 110:645-655.

Hubbell, J.A. 1995. *Biotechnology (NY)* 13:565-76.

Jones, M.K., H. Wang, B.M. Peskar, E. Levin, R.M. Itani, I.J. Safeh, and A.S. Tarnowski. 1999. *Nature Medicine* 5:1418-23.

Krieg, A.M. 1999. *J. Gene Med.* 1:56.

Krieg, A.M., S. Matson, T.J. Waldschmidt, G.A. Bishop, R. Teasdale, G.A. Koretzky, and D.M. Kinman. 1995. *Nature* 374:546.

Labhasetwar, V., J. Bonadio, S. Goldstein, W. Chen, and R.J. Levy. 1998. *J. Pharm. Sci.* 87:1347.

Lamberg, A., T. Helaakoski, J. Myllyharju, S. Peltonen, H. Notbohm, T. Pihlajaniemi, and K.I. Kivirikko. 1996. *Journal Biol. Chem.* 271:11988.

Lauffenburger, D.A., and D.V. Schaffer. 1999. The matrix delivers. *Nature Medicine* 5 (7) 733-734.

Lin, P.H., D. Ren, M.K. Hirko, S.S. Kang, G.F. Pierce, and H.P. Greisler. 1998. *Atherosclerosis* 137(2):227-289.

Magari, S., V. Rivera, J. Luliucci, M. Gilman, and F. Cerasoll, Jr. 1997. *J. Clinical Invest.* 100 (11):2865–2872.

Montesano, R. 1992. *Eur. J. Clin. Invest.* 22:506.

Morris, E. 2000. In *WTEC Workshop on Tissue Engineering Research in the United States, Proceedings, June 5, 2000*. Baltimore, MD: International Technology Research Institute, Loyola College

Otsuka, Y., H. Mizuta, K. Takagi, K. Iyama, Y. Yoshitake, K. Nishikawa, F. Suzuki, and Y. Hiraki. 1997. *Developmental Growth Differential* 39:143-156.

Pineda, S., A. Pollack, S. Stevenson, V. Goldberg, and A. Caplan. 1992. *Acta Anatomy* 143:335-340.

Sakiyama, S., J. Schense, and J. Hubbell. 1999. *FASEB J.* 13:2214-2224.

Sakiyama-Elbert, S., and J. Hubbell. 2000. *J. of Controlled Release* 100:389-402.

Santini, J.T., M.J. Cima, and R. Langer. 1999. *Nature* 397:335.

Schense, J., and J. Hubbell. 1999. *Bioconjugate Chem.*10 (1):75-81.

———. 2000. *The American Soc. for Biochemistry and Molecular Biology, Inc.* 10:6813-6818.

Shea, L.D., E. Smiley, J. Bonadio, and D.J. Mooney. 1999. *Nature Biotech.*17:551.

Shireman, P.K., B. Hampton, W.H. Burgess, and H.P. Greisler. 1998. *Surgical Forum* 49:348-350.

Shireman, P.K., L. Xue, E. Szylobryt, W.H. Burgess, and H.P. Greisler. 2000. *J. Vasc. Surg.* 31:382-390.

Simons, M. 2000. Angiogenic Factors. In *WTEC Workshop on Tissue Engineering Research in the United States, Proceedings, June 5, 2000*. Baltimore, MD:International Technology Research Institute, Loyola College.

Takahama, Y., T. Ochiya, H. Tanooka, H. Yamamoto, H. Salamoto, H. Nakano, and M. Terada. 1999. *Oncogene* 18 (43):5943-5947.

Truong-Le, V, August J.T., and K. Leong. 1998. Controlled gene delivery by DNA-gelatin nanospheres. *Human Gene Therapy* 9:1809-1717.

Vuorela, A., J. Myllyharju, R. Nissi, T. Pihlajaniemi, and K.I. Kivirikko. 1997. *EMBO J.* 16:6702.

Xue, L., P. Shireman, B. Hampton, W.H. Burgess, and H.P. Greisler. 2000. *J. Surgical Research* 92:255-260.

Xue, L., A.K. Tassiopoulos, S. Woloson, D.L. Stanton, Jr., C. Sullivan, B. Hampton, W.H. Burgess, and H.P. Greisler. In Press. *J. Vasc. Surg.*

Yamada, K.M. 1991. *J. Biol. Chem.* 266:12809-12812.

Ye, X., V. Rivera, P. Zoltick, F. Cerasoli, M. Schnell, G. Gao, J. Hughes, M. Gilman, and J. Wilson. 1999. *Science* 283:88.

CHAPTER 5

CELL-BASED TECHNOLOGIES: NON-MEDICAL APPLICATIONS

Milan Mrksich

INTRODUCTION

This chapter provides an overview and regional comparison of the development of cell-based technologies for applications that lie outside of tissue engineering. Efforts to integrate cells with conventional microtechnologies are motivated by the promise of extending the capabilities of current microsystems and of providing technical solutions to unfulfilled applications. Cell-based sensors capable of detecting and identifying biological warfare (BW) agents represent the first examples of hybrid microsystems that combine living and non-living materials. Conventional approaches are not yet capable of creating unattended sensors that can selectively detect pathogenic viral and bacterial agents. The integration of cells—which are the natural targets for these agents, and hence can respond to their presence—with microsystems that can interrogate the biological status of the cells, now provides a route to BW sensors. In other examples, cells may augment today's microsystems technologies by providing energy, actuation, or even computational processes.

Why is this topic included in a global assessment of tissue engineering? First, the field of tissue engineering provides the intellectual platform and technological infrastructure for engineering devices that combine cellular and materials components. Research in tissue engineering has revealed the design rules for joining cells with materials and for understanding the mechanisms by which cellular functions can be influenced or interrogated by materials. Further, many researchers in this emerging field either have training in tissue engineering or collaborate closely with the tissue engineering community. Second, development of cell-based microsystems outside of tissue engineering will, in the long term, provide technologies that will be applied to tissue engineering. The technology developed to integrate the functions of cells with electrical or mechanical processes in materials will, for example, have important benefits to the growth of tissue for transplantation and for prosthetic interfaces between indwelling devices and natural tissue.

The field of cell-based engineering is at a very early stage, with a small number of researchers in each geographic region addressing aspects of cell-materials integration. Although the field is not yet a recognized area of research and development, recent successes with cell-based sensors have prompted increased activity that will likely continue over the next five- to ten-year period to establish a sustainable R&D activity. It follows that the observations and conclusions outlined in this chapter represent an early assessment of this field, which will likely see substantial changes over the next several years. Unlike the other topics covered in this WTEC study, no geographical region has established a critical base in cell-based engineering that ensures a dominant position as this technology matures.

Scope of Cell-Based Engineering

Cell-based engineering addresses the development of hybrid devices that combine cellular and tissue components with conventional materials and processes found in microfabrication. Research and development activities span a broad range of topics, including technical development of methods and

fabrication routes to join cells with materials; exploratory and discovery research to identify strategies for matching cellular processes with materials processes; and engineering of complete systems that exploit the unique performance of cell-based devices for new applications. The recent activity is motivated by the realization that combining man-made systems and biological systems, each of which has unique characteristics, could yield engineered devices with broad new capabilities. In the near term, a central challenge in these programs is the development of a common framework for designing and building structures having both materials and biological components. This framework must address the development of strategies to integrate the functions of *engineered systems*, which are based on firm physics and engineering, use inorganic and metallic materials, and are constructed with photolithography and microfabrication tools, with the functions of *biological systems*, which use soft materials in aqueous environments, rely on self-assembly for their construction, and where the design rules are in many cases incompletely understood.

This WTEC study grouped cell-based microsystem activities into three areas, described below. Other applications that use cellular properties in microsystems—including the supply of energy and control of mechanical elements—remain uninvestigated and are not included in this chapter.

1. *Cell-based sensors*. These represent the most advanced and commercially viable example of cell-based engineering.
2. *Neural networks*. In these, neural cells are patterned on microelectrode arrays and are under active development as possible computational elements. While still far from a demonstrated application, this work is providing the basis for implementing new computational architectures.
3. *Dynamic and responsive interfaces*. In these, cellular activities can be either influenced or interrogated by electrical processes in a contacting substrate, represents an exploratory activity with expected long-term technological opportunities.

An overview follows of these three areas, including regional comparisons. Greater detail can be found in the site visit reports in Appendices B and C and in the summary of the June 2000 U.S. review workshop in Appendix D.

OVERVIEW OF R&D ACTIVITIES

Cell-Based Sensors

Sensors of chemical and biological agents, including viral and bacterial pathogens, are important to clinical diagnostics, food monitoring, and detection of biowarfare agents in urban and military settings. Yet current sensors still lack the combination of selectivity, sensitivity, and fast response time needed for many applications; they fall far short for real-time sensing with hand-held devices. Cells and tissues have several characteristics that make them well suited for sensing biological targets. Cells present multiple receptors on their surfaces (some of which have low specificity for single targets) and rely on complex nonlinear information processing that allow them to identify agents with high accuracy. Cells also employ amplification schemes to improve sensitivity and reduce response times. The use of cells as sensor elements still requires that the cells be joined with a materials device and that the natural transduction mechanisms of living cells be translated to give electrical outputs from the device.

One approach uses microelectrode arrays to monitor ion channel activity in adherent neuronal cells. This strategy is well suited for detecting neurotoxins and other chemical agents that act against membrane channel receptors. Several research groups have developed and fabricated integrated arrays that are tailored to these applications and have developed microfluidic cassettes that permit automated sample introduction and assays. There have also been important advances in developing pattern recognition systems that can identify with better accuracy the source of changes in electrical activity. The United States is the leader in developing integrated, cell-based devices that combine sophisticated electrical and microfluidic engineering (Stanford University and the Naval Research Laboratory). It is noteworthy that these programs have emphasized engineering considerations and have not yet made use of sophisticated cell and molecular biology to engineer cells that respond to a broader class of agents and do so with greater specificities. Significant work in Europe, by contrast, is principally aimed at fundamental studies of electrical activities in neuronal cultures

and has not yet targeted cell-based sensors (University of Glasgow and Max Planck Institute). The strong background of European researchers in electrophysiology, particularly at the Institute of Neurophysiology at Koln, would prove an important asset in applied research.

A second approach has used cells that are engineered to give spectroscopic signals in response to specific signal transduction pathways. Most strategies use cells that are transfected with green fluorescent protein (GFP) and can be applied to sensors of a vast array of target analytes. Cells that are engineered to express the GFP under the control of specific promoters report on the gene expression that is associated with specific cellular processes. In some strategies, cells are engineered such that GFP fusion proteins undergo translocation within the cell, for example, localization of transcription factors from the cytosol to the nucleus. Other strategies rely on fluorescence energy transfer between pairs of chromophores. This class of cell-based sensors offers wide flexibility in engineering cells to respond to a range of targets because they give direct information on key molecular processes within the cell. There have also been important advances in informatics (see Chapter 7), including the development of software architectures for storing and mining fluorescence data in order to give robust identification of targets. The United States is the most advanced in developing and commercializing cell-based sensors for both drug discovery programs and detection of biological warfare agents (Cellomics), but Europe and, to a lesser extent, Japan have active programs to develop new strategies by which cells can be engineered to report on biological activities.

Neural Networks

Integrated circuits and brain tissue both perform complex computations, but each is based on exceedingly different materials, designs, and processes. There has been a long-standing interest in understanding the schemes by which information is processed in the brain and in using cultured neuronal arrays to mimic these processes. Fusing tissue processes—or the designs that are inherent to these processes—with current integrated circuit technology could provide devices that combine the high speed and memory of chips with the pattern-based computation and adaptability of neural tissue.

Current programs in neural networks have addressed a range of technical and materials issues that are needed for building the neuronal arrays, but they have not yet moved to exploring the properties of these arrays and assessing their potential to perform computation. Important work has developed a portfolio of methods for patterning the positions of neuronal cells on planar substrates and controlling the positions of functional synapses between neighboring cells. Related work has developed the microelectrode arrays that are used to address electrical processes in the cellular networks. Several innovations have been important to optimizing the interface between cells and electrical elements to provide for efficient stimulation and recording of electrical activities from populations of neurons. These advances now enable the reproducible and controlled preparation of neuronal arrays that are interfaced with microelectrode arrays (Figure 5.1). Efforts over the next few years will investigate simple computational processes in the neural networks and will inform the further development of these constructs for appropriate applications.

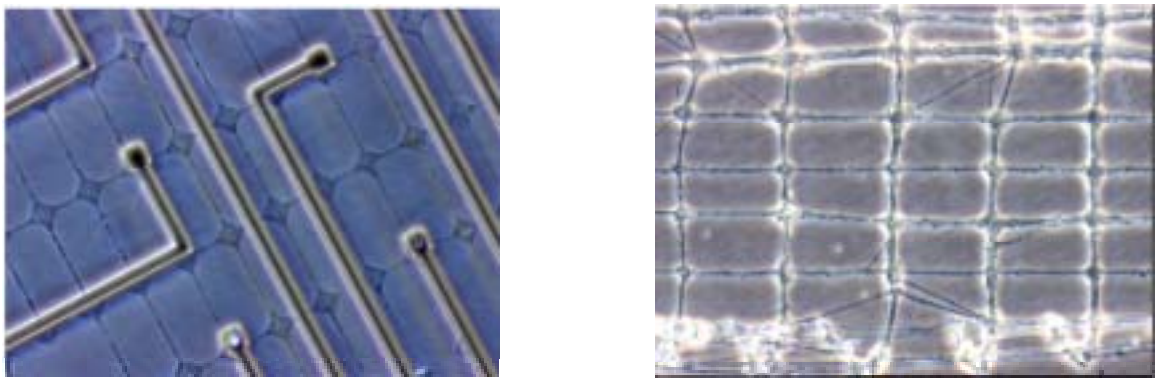


Fig. 5.1. (*Left*) Example of a microfabricated substrate that combines an array of electrical elements with a pattern of polymer that directs the positions and connections of neural cells. (*Right*) Image of a population of neuronal cells that are patterned on the substrate. The cells assemble functional synapses and display coordinated electrical activities.

Dynamic and Responsive Interfaces

On a broader level, the integration of cells and tissues with materials requires new strategies for fusing biological processes and materials processes. The development of a variety of strategies for transducing biological and electrical signals will create a platform for designing hybrid devices that truly integrate living and nonliving components. The first examples of dynamic interfaces have been reported only in the last three years and still represent exploratory research efforts. An impressive program in Japan is developing thermally responsive interfaces and applying these to generation of complex cell sheet structures (Tokyo Women's School of Medicine). This work is based on poly(acrylamide) layers that undergo expansions and contractions with changes in temperature and therefore can be switched between states that permit or prevent cell attachment. These engineered surfaces have been patterned to prepare cellular co-cultures and to enable the nondestructive harvesting of cultured cells and tissues.

Programs in Europe (e.g., at ETH) are developing responsive materials whose interactions with cells change over time due to cellular enzymatic activities. Work in Professor J.A. Hubbell's laboratory is creating materials that mimic the enzymatic processes that underlie cellular remodeling of protein matrices. These new materials blur the distinction between biological and synthetic components and offer new opportunities to interface cells with synthetic matrices. Work in the United States is developing electroactive substrates that can modulate the presentation of ligands to an attached cell and hence control cell behavior in real time (University of Chicago, Figure 5.2). The strategies utilize a molecular engineering approach to creating ligands whose activities can be turned on or off by the application of electrical potentials to the underlying substrate. Taken together, these early examples provide demonstrations that materials can be engineered in ways that offer a more biologically relevant interface to cells and tissues. This work provides new strategies that can be brought to the design and fabrication of cell-based devices.

Fig. 5.2. Example of a dynamic substrate that can be electrically switched to turn on cell migration. Cells were initially patterned on circular regions and maintained in culture (*left*). Application of an electrical potential to the substrate switched the surrounding regions to a state that promoted cell migration.

SUMMARY

Technical Status

General comparisons of the expertise of the United States, Europe, and Japan in several cell-based engineering themes are discussed below and summarized in Table 5.1. It is important to recognize that the comparisons are based on very early activity in each region and hence will likely change over the next several years.

Cell-Based Sensors

The United States maintains the dominant position in cell-based sensors. The principle motivation and resources to advance this technology have come from the Department of Defense for development of sensors for pathogenic agents and from the capital markets for development of screening tools used in drug discovery.

Table 5.1
Comparisons Between the United States, Japan, and Europe in Cell-Based Technologies and Nonmedical Applications

Cell-based sensors	U.S. holds dominant position
Neural networks	Excellent programs in U.S., Europe, Japan
Other applications (pumps, power sources, microfabrication)	Little progress anywhere
Engineering active interfaces	Limited but excellent work in all regions

Neural Networks

Europe, Japan, and the United States maintain a comparable position in developing neural networks. Work to date has addressed several technical needs for patterning cells, fabricating microelectrode arrays that are compatible with cell culture, and optimizing the interface between cell and material to permit efficient electrical communication. Work in the next period will characterize the properties of these networks and begin to define appropriate applications.

Other Applications

Very little, if any, effort has been directed towards demonstrating additional functions that cells bring to microsystems. Efforts in the next period will likely explore the use of cells to provide energy to microsystems and to serve as mechanical elements in actuation and pumping.

Engineering Active Interfaces

Each region has demonstrated early examples of active interfaces between cells and materials. These examples, which suggest entirely new ways of integrating the functions of cells with electronics, are certain to motivate a much wider R&D effort, with potentially significant outcomes.

Relative Strengths

Programs to engineer cell-based devices must combine expertise from a wide range of technical areas. Relative strengths of each area are indicated below. These ratings do not address the state of a technical area in a region, but rather they reflect the present importance of the area in cell-based engineering programs.

Engineering

Programs in both Japan and the United States reflect a strong engineering base. Many of the research teams are led by engineers and utilize sophisticated microfabrication processes. Work in these regions, particularly in commercialization of cell-based sensors, has gone beyond research and development and has emphasized the development of integrated systems.

Materials

Programs in all three regions share a strong technical position in materials. Strengths include chemical and physical approaches to surface modification in order to promote cell adhesion, ensure biocompatibility, and provide for dynamic interactions between cells and materials.

Biology

Programs in Europe and the United States make frequent use of molecular and cell biology techniques. The most important use is genetic engineering to provide cells that can selectively sense biological or chemical agents. There is, however, still a large unexploited opportunity to engineer cells that can interact in selective ways with the materials to which they are attached.

Industrial Influence

The United States is the leader in providing opportunities and capital for commercialization of emerging technologies. Europe has, over the past few years, made significant progress in this area and now has a significant portfolio of startup technology companies. Japan has not yet implemented a strategy for technology transfer into small, entrepreneurial companies.

Funding Mechanisms

Research and development efforts in all three regions suffer from a lack of targeted federal investment in university laboratories. Much of today's work in cell-based engineering is supported by special nonrecurring programs and, when the work is related closely to a possible application, by private capital. The lack of funding for cell-based engineering remains a significant obstacle to the growth of this area; there is, therefore, an opportunity here for regions that provide targeted investment.

Key Factors for Future Development

Several factors that are important to developing a broader program in cell-based engineering are summarized below. It is particularly important to promote the extensive level of collaboration that is required in these efforts.

Multidisciplinary Teams

Programs in cell-based engineering require an unprecedented need for collaboration between engineers, biologists, chemists, physicists, and informatics researchers. The need will be met most effectively when researchers from distinct backgrounds assemble into teams rather than rely on multiple collaborations between teams. In practice, the organization of multidisciplinary teams is not possible in many research institutions.

Institutional Culture and Infrastructure

Programs in cell-based engineering will benefit from institutional environments that promote a culture of multidisciplinary interaction, wherein researchers and engineers from disparate areas work together and adopt a common language. Further, institutions must provide facilities that are equipped for the range of experimental work required in cell-based engineering and allow researchers from different departments and divisions to share that space. Small companies have been the most successful in these respects; they may serve as a model for university and government laboratories.

Fundamental and Applied Research

A mature program in cell-based engineering must maintain a balance between applied efforts and basic research. The former delivers commercial technologies, whereas the latter furnishes a constant stream of new opportunities for commercialization. While cell-based sensors represent an early example of a commercially viable technology, there is currently an inadequate level of basic research to sustain further development.

Cross-Training of Researchers

As with all new technologies, the development of an R&D infrastructure will be limited by the availability of suitably trained personnel. Universities are the most effective at training skilled personnel but will require revised curricula and new support for research programs in this area. Currently, tissue engineering research groups provide an excellent training ground.

Targeted Federal Support

The number of research and development groups involved in cell-based engineering is small but poised to grow significantly over the next five- to ten-year period. The growth will in large part be dictated by federal support of R&D activities in academic, government, and commercial laboratories. Federal support of

cell-based engineering is likely the single most important factor in determining which region assumes a leadership area and has the best opportunities for building a commercial technology.

Observations and Conclusions

Early Stage Technology. The field of cell-based engineering is at a very early stage of development. Many of the technical manipulations that underlie this field have been developed over the past twenty years; efforts to utilize cells to extend the functions of microsystems and to target specific applications have been initiated only within the past five years. Hence, no region holds a dominant position in this emerging technology. Europe, Japan, and the United States each have a strong beginning in the area, but no one region has provided for a growing effort that sustains itself over the longer term. With the proper emphasis and resources, any region can assume a leading role in developing a research infrastructure and in translating that base to a broader commercial activity.

The United States Has an Advantage in Modes for Collaboration

The culture of collaborative and entrepreneurial research in the United States is a significant advantage in building the research teams and environments that promote multidisciplinary and collaborative research programs. Campus institutes that are created to bring together researchers and engineers from various departments to address an emerging technical theme (for example, genomics and proteomics) are now common in the United States. In particular, institutes that pair engineers, materials chemists, and biologists are emerging and provide an ideal environment for the growth of cell-based engineering. While Europe and Japan each have a limited number of centers that integrate the disciplines, significant obstacles in academic institutions hinder such efforts.

Industrial-Academic Ties are Important

The United States remains the single leader in commercialization of new technologies. Cell-based sensors, for example, are now a commercial technology in the United States. The leading U.S. position is due to a combination of clear pathways for patenting and licensing in the universities, an active interest in commercialization opportunities by academic researchers, and a sophisticated and well-capitalized private investment community. While Europe and Japan recognize the importance of developing the infrastructure for commercialization of research, they are only beginning to see successful examples of new ventures.

Strong Synergies with Tissue Engineering

The researchers and the technical approaches that are used in cell-based engineering have substantial overlap with the tissue engineering community. This overlap provides a synergy that will be important to supporting the development of a broader cell-based engineering effort and to providing sophisticated technologies to tissue engineering in the longer term. Strategies to move towards a broader cell-based engineering effort should emphasize this close synergy with tissue engineering.

Unanticipated New Technologies

New technologies that emerge from connections between traditionally separate fields are often difficult to anticipate prior to exploratory and discovery research. While the use of cells as sensing elements in engineered microsystems is now established and is in commercialization, there is little consensus on what other applications will be best served by cell-based devices. What is certain is that both of the parent technologies—biotechnology and microelectronicsare commercially viable and that a hybrid technology that combines appropriate characteristics of each parent will provide capabilities that are simply not available today.

CHAPTER 6

ENGINEERING DESIGN ASPECTS OF TISSUE ENGINEERING

David Mooney

INTRODUCTION

Tissue engineering is rapidly evolving from the initial proof-of-principle demonstrations of feasibility to the development of products intended for widespread clinical use. A number of critical engineering design issues (Figure 6.1) must be addressed during this transition to enable large-scale manufacture and use of a variety of engineered tissues. These challenges include elements of mass transport, biomechanics, biomaterials, and bioelectronics. Biomaterials and bioelectronic issues are covered in other chapters of this report. Important engineering design issues addressed in this chapter include

- adaptation of existing bioreactor technology for large-scale cell expansion and three-dimensional tissue production
- identification of appropriate techniques (e.g., cryopreservation) for preserving both cells and engineered tissues (cytopreservation)
- development of strategies to promote vascularization of engineered tissues (mass transport issues)
- evaluation of the critical mechanical properties of the tissues that need to be replaced
- determination of the minimum values of native tissue mechanical properties required of an engineered tissue
- exploitation of externally applied mechanical stimuli to regulate the development and function of engineered tissues

Significant progress has been made in the United States, as compared to Europe and Japan, in addressing many of the bioreactor issues. However, significant progress will be required in both the cryopreservation and vascularization areas to achieve the full potential of tissue engineering products. The importance of the biomechanics issues are just now being fully recognized, and this is an underdeveloped area. Significant progress in all of these areas is critical to efforts to engineer functional tissues that can exist in a mechanically dynamic environment (e.g., bone cartilage, blood vessels).

A brief review of each topic is given in the following sections. More information regarding specific efforts at different sites can be found in the site reports (see Appendices B and C). A tabular summary is given in the final section. For the tissue engineering field to reach its potential, there are clearly critical requirements for advances in several areas.

BIOREACTOR TECHNOLOGY

Bioreactors are utilized in tissue engineering for a variety of diverse applications (Miller 2000):

- cell production on both a small, individual patient and a large, multipatient scale
- production of three-dimensional tissues *in vitro*
- directly as organ support devices

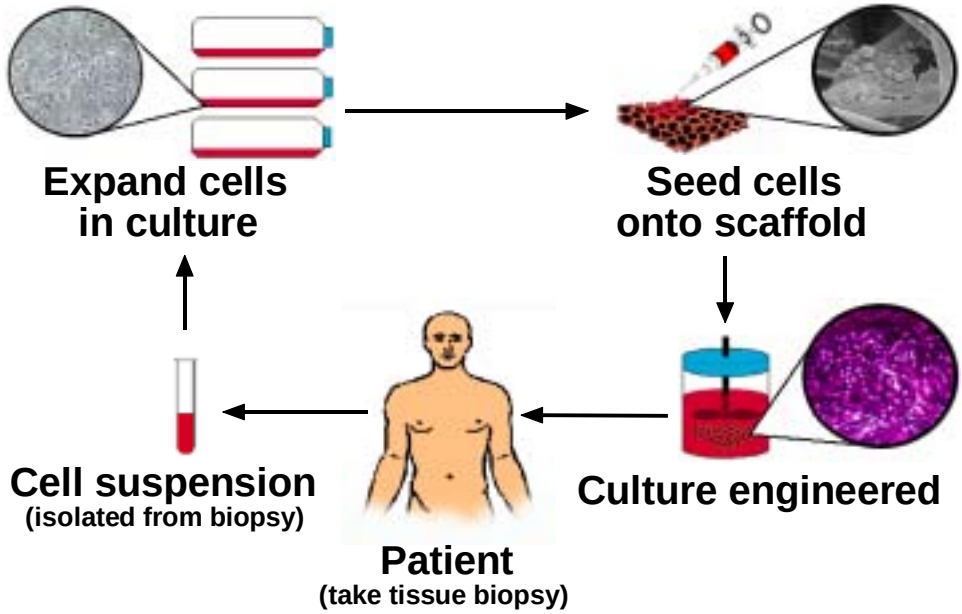


Fig. 6.1. The typical tissue engineering approach demonstrates multiple engineering design issues that must be addressed. Cells are expanded from a tissue source, requiring bioreactor technology. Following combination with a biomaterial, three-dimensional engineered tissues are often cultured for a period of time *in vitro*, again requiring bioreactor systems. Storage of cells and tissues prior to transplantation requires appropriate preservation, and the survival and function of tissues following implantation requires vascularization from the host in most situations. In addition, the mechanical properties of the engineered tissue (e.g., cartilage, blood vessels) must be appropriate if it is to suitably replace tissue function (M.C. Peters, U. Michigan; used by permission).

Cell Expansion

Many tissue engineering strategies rely on multiplying cells from a small biopsy or initial tissue source and subsequently harvesting these cells for transplantation directly or on a polymeric scaffold. Currently, efforts in both Japan and Europe are focused on the use of autologous cell therapies, and a large number of their academic centers and companies are developing autologous tissue engineering products. These include the Japan Tissue Engineering Co. and Riken Cell Bank in Japan; Cell Lining GmbH (Germany); Imperial College (UK); Valley Tissue Engineering Center (Germany); and Biomaterials and Tissue Repair Inserm-U.443 (France). In contrast, both autologous and allogeneic therapies are being pursued in the United States. Representative U.S. companies that have commercialized allogeneic cell-based products include Advanced Tissue Sciences (La Jolla, CA), and Organogenesis (Canton, MA). U.S. companies that utilize autologous cell therapies include Genzyme Tissue Repair (Cambridge, MA), Curis (Cambridge, MA), and Aastrom (Ann Arbor, MI).

Allogeneic products are amenable to large-scale manufacturing at a single central site, while autologous therapies will likely lead to more of a service industry, with a heavy emphasis on local or regional cell banking/expansion. Previous bioreactor technologies, which focused on growing single cells or small cell clusters, provide a suitable starting point for both the autologous and allogeneic types of cell expansion work. However, different technologies will likely be optimal for the two approaches . The local or regional cell expansion required for autologous therapies will require robust, mobile cell multiplication systems. However, European and Japanese sites do not appear to be focused on developing new bioreactor

technologies, but are adapting established processes. Only one company known to the panelists (Aastrom) has focused on this issue.

Three-Dimensional Tissue Culture

Production of three-dimensional engineered tissues *in vitro* for subsequent transplantation is a major emphasis in many tissue engineering companies and academic laboratories. This process typically involves culture of cell-biomaterial constructs following seeding of previously expanded cells (see previous paragraphs) onto the three-dimensional scaffold. Engineered skin products, some of which are available for clinical use and others in late-stage clinical trials (Naughton 1999; Parenteau 1999), are an example of this approach. Several U.S. companies have developed large-scale tissue production facilities, with the goal of reproducibly producing large numbers of individually packaged tissues.

Bioreactors as Organ Support Devices

Cell-containing bioreactors are also used directly as support devices for liver (bioartificial liver, BAL) (Figure 6.2) or kidney (bioartificial kidney, BAK) function. The BAK is proposed as an adjunct or replacement to dialysis for patients with kidney failure. The BAL devices may be useful as a bridge to transplantation in cases of irreversible liver failure or as a bridge to restoration of liver function in situations of acute liver toxicity (Tabata 2000). This concept has been pursued for several years by a number of U.S. academic groups and companies (e.g., Circe Biomedical of Lexington, MA and Hepatix of La Jolla, CA). Due to societal limitations on liver transplantation, this technology is of great interest in Japan. Several research groups have active programs in this area, including Dr. Oshima's group at the University of Tsukuba, Dr. Iwata's group at Kyoto University, and Dr. Akaike's work at the Tokyo Institute of Technology. In Germany the Virchow/Hybrid Organ GmbH is also attempting to develop and commercialize a BAL system. For both the BAL and BAK, transport between the cells in the device and fluids flowing through or in partial contact with the contained cells must be optimized (McLaughlin et al. 1999; Nikolovski et al. 1999), as the utility of these devices is completely dependent on this function (e.g., clearance of toxic metabolites in blood). A large number of BAL designs have been developed in an effort to optimize this process while minimizing the device volume (McLaughlin et al. 1999), while a lesser amount of work has been done to date with the BAK.

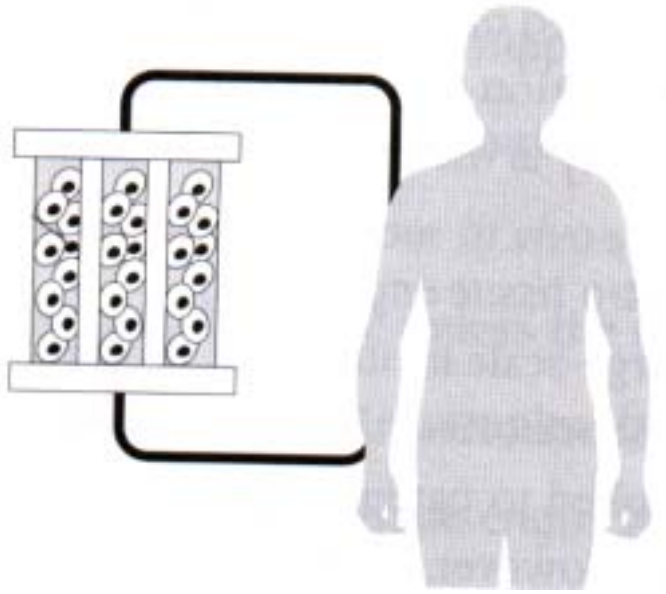


Fig. 6.2. Bioartificial liver support device. These bioreactor types, which contain liver-cells, are used as extracorporeal support devices for temporary replacement of liver function. The blood, or plasma, of a patient in liver failure is circulated through the device. Inside the device, the liver cells clear toxic substances from the patient's blood (Tabata 2000).

Summary

Many different types of bioreactors have been developed for the diverse bioreactor applications in tissue engineering. Ideally they must all allow for control over the physicochemical environment (e.g., pO_2, pH, PCO_2, shear rate), allow aseptic feeding and sampling to follow tissue development, and maximize use of automated processing steps to increase reproducibility. Standard bioreactor technologies are well suited to address many of these issues for cell expansion, but they have limitations when used for the other tissue engineering applications (Miller 2000). In particular, the cultivation of three-dimensional tissue constructs and use of bioreactors for BAL and BAK applications place great demands on the mass transport function (e.g., nutrient distribution), and this is the basis for significant research (Obradovic et al. 1999). In addition, it may be necessary to simultaneously culture multiple cell types for certain applications, and this may require more complex bioreactor designs (Emerson et al. 1991).

PRESERVATION OF CELLS AND ENGINEERED TISSUES

Cells, macromolecular biologically active drugs, and three-dimensional tissues grown in bioreactors will all likely be important tissue-engineering products. In all three cases, it will be critical to develop technologies for the stable storage of these products following production and prior to clinical utilization. Cryopreservation, as compared to cold storage, potentially affords long shelf life, low risk of microbial contamination, and cost effectiveness (Karlsson and Toner 2000). This type of storage typically involves reducing or removing water (e.g., lyophilization of protein solutions). The controlled transport of water from the proteins, cells, and tissues is a complex mass transfer problem. Long-term storage of protein products is an important issue that has received extensive attention in the biotechnology and pharmaceutical industries (Wang 2000). Effort is also being devoted to develop appropriate cryopreservation processes for DNA-based therapeutics (Anchordoquy and Koe 2000). Cryopreservation of cell suspensions is routine for many cell types, but tissue cryopreservation is still an emerging field with many challenges (Karlsson and Toner 2000). An inability to image the process of tissue freezing is one of several challenges (Bischof 2000). Autologous-based cell products that are produced locally or regionally likely will not require long-term tissue storage, and cold preservation may be adequate. However, large-scale allogeneic tissue production would benefit significantly from the development of techniques that could allow long-term tissue storage.

MASS TRANSPORT ISSUES FOLLOWING IMPLANTATION

There are at least two critical mass transport issues following implantation of an engineered tissue. First, it is critical that transplanted cells or engineered tissues have sufficient nutrient and waste exchange with their surroundings in order to survive, function appropriately, and become integrated with host tissue following implantation. Oxygen transport is typically considered the limiting factor for nutrient exchange (Colton 1995) (Figure 6.3). Secondly, in immunoisolated cell therapies the membrane must not be a barrier to diffusion of desirable molecules (e.g., oxygen, therapeutic molecules secreted by transplanted cells) while blocking diffusion of undesirable species (e.g., elements of the host immune response).

Vascularization

Tissues in the body overcome issues of oxygen and nutrient distribution by containing closely spaced capillaries that provide conduits for convective transport of nutrients and waste products to and from the tissues. It is similarly considered critical for any engineered tissue of significant size to become vascularized, with the exception of cartilage. Several approaches are currently being investigated to promote vascularization of engineered tissues. First, scaffolds utilized for cell transplantation are designed to promote invasion of host fibrovascular tissue by the inclusion of large, interconnected pores (Mikos et al. 1993). However, fibrovascular ingrowth into the scaffolds occurs at a rate less than 1 mm/day and typically takes one to two weeks to completely penetrate even relatively thin (e.g., 3 mm thick) scaffolds. The second, more active, approach to promote vascularization of engineered tissues is the delivery of angiogenic growth factors (e.g., VEGF, bFGF) to the implant site. It has recently been demonstrated that these factors may be directly included within the tissue engineering scaffolds for a sustained delivery at the desired site (Tabata 2000; Sheridan et al. 2000).

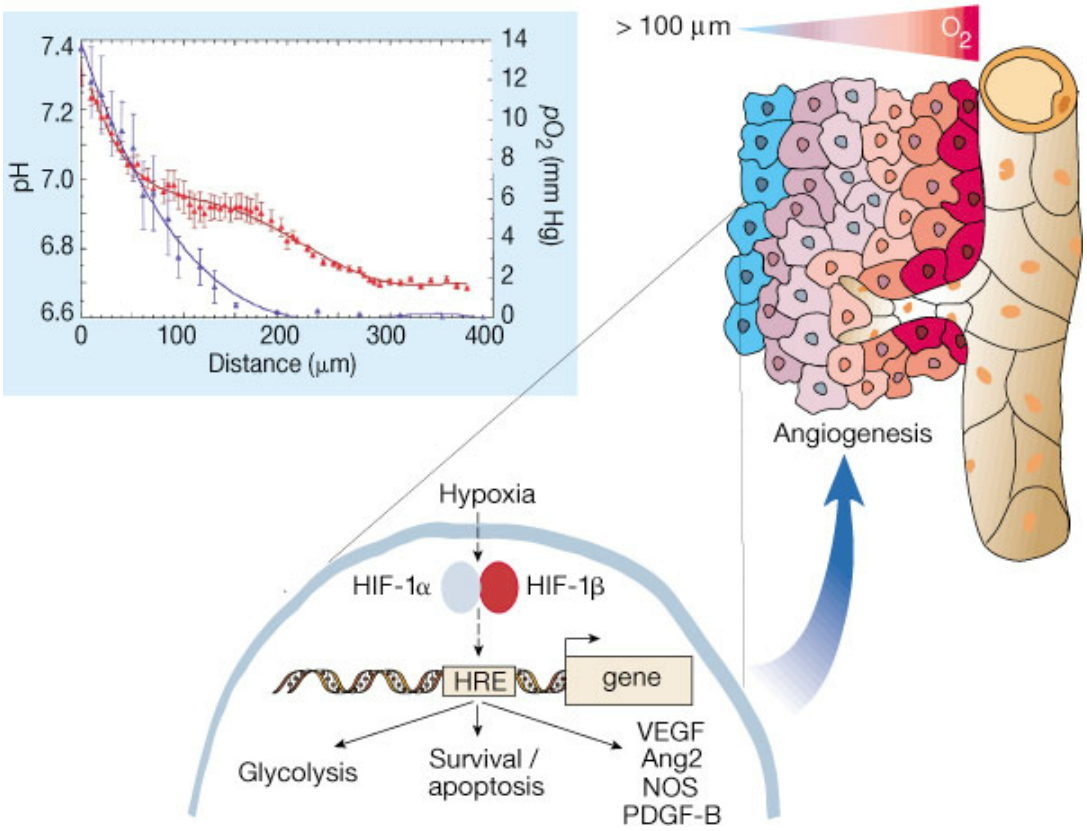


Fig. 6.3. Illustration of rapid depletion of oxygen provided by a capillary as it diffuses into and is consumed by cells in the surrounding tissue (cartoon on upper right). Oxygen is effectively depleted within 100-200 microns of a capillary in most tissues, and the pH also falls significantly in this distance (upper left graph). Hypoxia within tissues lacking sufficient vascularization can lead to up-regulation of genes that encode for angiogenic molecules (e.g., VEGF) (lower middle cartoon); however, this will not likely lead to increased vascularization in a time frame consistent with survival of cells transplanted in a large device(from Carmeliet and Jain 2000).

Other vascularization strategies are being explored as well. It may be possible to utilize local gene therapy to promote vascularization by delivery of plasmid DNA, which encodes the growth factors from the tissue-engineering scaffold (Fang et al. 1996; Shea et al. 1999; Ochiya et al. 2000). The majority of protein and DNA delivery strategies focus on release of the factors from polymeric scaffolds to allow for their diffusion into surrounding tissues. In contrast, some groups (e.g., A. Goepferich's group at Regensburg University in Germany) are instead covalently coupling these factors to the polymer scaffold. This approach will specifically target cells in contact with the scaffold. Another approach to promote vascularization is to transfect the cells to be transplanted with genes encoding for angiogenic molecules (Ajioka et al. 1999). A third approach to enhance angiogenesis in engineered tissues is to co-transplant endothelial cells along with the primary cell type of interest. The endothelial cells seeded into a tissue engineering scaffold form capillaries that can merge with capillaries growing into the scaffold from the host tissue (Nor et al. 1999). This may increase the rate and extent of vascularization of engineered tissues.

A long-term goal of tissue engineering is to grow large-three dimensional tissues (e.g., a complete liver) in culture for subsequent transplantation. To be successful in this approach it will be necessary to develop a pseudo-vascular network in the tissue. This network would be perfused with medium in culture to enable appropriate nutrient distribution throughout the tissue volume, and anastomosed to the native blood supply following implantation to meet the same requirement *in vivo*. This is clearly an ambitious goal, but several research groups (e.g., J.P. Vacanti at Harvard Medical School and H. Iwata at Kyoto University in Japan) have begun efforts to address this possibility.

Immunoisolation

In certain tissue-engineering applications the function of transplanted cells is purely biochemical (e.g., secretion of a protein for local or systemic distribution). In this situation it may be possible to transplant xenogeneic or allogeneic cells without host immunosuppression, if the cells can be isolated from the host immune system. Polymeric membranes are often utilized in these situations (Lysaght et al. 1994). However, cells in the devices must survive by diffusion of nutrients from the surrounding host tissue, and this limits the maximum size of these devices to diameters less than 1 mm (Colton 1995). The constraint imposed by mass transfer limitations has led to several device designs that attempt to balance maximum diffusional transport potential without compromising other functions of the device such as mechanical stability (Lysaght et al. 1994). In any design, however, the numbers of cells that can be delivered in any practical system are limited, and this approach is only appropriate when relatively few cells (e.g., millions) need to be delivered. However, this may not be a limitation for many important clinical applications, potentially including diabetes and central nervous system applications (Sun et al. 1996; Bachoud-Levi et al. 2000). A critical engineering design issue in this area is the lack of data regarding the relationship between barrier permeation properties and immunoisolation effectiveness. Furthermore, widely differing degrees of success have been reported by various groups, perhaps relating to immunological or mass transport issues specific to each application and device design (Colton 2000).

BIOMECHANICS ISSUES

Many of the tissues for which one may desire to engineer a replacement have a mechanical function(s), including blood vessels, bone, and cartilage. However, the mechanical properties of many tissues engineered to date are inferior to those of native tissues (Cao et al. 1994; Carver and Heath 1999; Kim et al. 1999; Niklason et al. 1999; Mauck et al. 2000; Seliktar et al. 2000). This finding clearly leads to two key biomechanics questions. First, what is the relevance of the mechanical properties of the engineered tissues to their function *in vivo*? Second, assuming the mechanical properties will be important, how can one control these properties of the engineered tissues? To address the first question, there will likely be several biomechanics aspects of native tissues that must be targeted. However, the mechanical properties of many of these tissues have not yet been precisely defined, and it is unclear which of the properties are important to use as design parameters for the engineered replacement tissues, and to what degree. It is relevant to the second question that externally applied mechanical signals are clearly regulators in the development and function of a variety of tissues. Increasing evidence from basic biology studies indicate cells mediate the response of tissues to mechanical signals, and the increasing amount of information available from these studies is now beginning to find utility in the design of engineered tissues.

Minimum Mechanical Properties Required of Engineered Tissues

In order to develop appropriate standards for the mechanical properties of engineered tissues it will be necessary both to understand the *in vivo* stress/strain in normal tissues in a variety of states, and to determine the complete mechanical properties of native tissues. There is considerable information available for certain tissues such as blood vessels and bone in the normal *in vivo* mechanical environment. However, for other tissues such as cartilage, there is a lack of data (Guilak 2000). Similarly, while there has been considerable effort to determine the mechanical properties of various tissue types, most biological tissues can be considered to be inhomogeneous, viscoelastic, nonlinear, and anisotropic materials (Guilak 2000). This complicates analysis of tissues, and the relationships between composition, structure, and mechanical properties of tissue are not completely defined.

At the current time it is unclear which of the many measurable tissue properties would be most important for specific engineered tissues, nor is it clear what minimum values for these properties would be appropriate for functional replacement. This issue is further complicated by the potential adaptation of engineered tissues to their mechanical environment following implantation. The limitations in the current knowledge base have been recognized by U.S. National Committee on Biomechanics, which has formed a subcommittee to provide an organized framework for addressing these issues. The principles underlying this endeavor have recently been outlined (Butler et al. 2000).

Mechanical Signals Regulating Cell Function

It has long been recognized that mechanical signals regulate the development of normal tissues, and a large number of investigators worldwide have been working to delineate the molecular mechanisms responsible for the response of individual cells to mechanical signals. For example, hemodynamic influences on the vascular system have been extensively studied (Konstantopoulos and McIntire 1997; Nerem 1993; Ando et al. 2000). There has been significant interest in identifying the role of specific cell-adhesion receptors in conveying this mechanical information into the cell (Ingber 1991; Shyy and Chien 1997), and in the complementary interactions between typical chemical-mediated (e.g., growth factors) signaling pathways and mechanical-mediated pathways (Giancotti and Ruoslahti 1999). These studies will likely define specific regimens of mechanical stimulation that optimally regulate gene expression in culture, and they may provide valuable input for mechanical stimulation of engineered tissues (see next section). In addition, delineation of the mechanisms by which mechanical signals regulate gene expression may ultimately provide new targets for intervention to regulate the structure and mechanical properties of engineered tissues.

Mechanical Signals Regulating Engineered Tissue Properties

A number of research groups, mainly in the United States, have recently begun to mechanically stimulate engineered tissues during *in vitro* development to determine if their mechanical properties may be modified with this type of input. The development of engineered skeletal muscle is clearly regulated by mechanical signals (Vandenburgh et al. 1991; Dennis and Kosnik 2000). The organization, composition, and function of engineered smooth muscle tissues and blood vessels can be readily modulated by application of physiologic regimens of cyclic strain (Niklason et al. 1999; Kim et al. 1999; Seliktar et al. 2000). For example, application of continuous cylic strain (7% amplitude; 1 Hz) leads to significant increase in the ultimate strength of engineered smooth muscle tissue, as compared to static cultured control tissues (Figure 6.4). Similarly, the mechanical properties of engineered cartilage can be improved by appropriate mechanical stimulation (Carver and Heath 1999; Mauck et al. 2000). These results are promising, but the properties of the engineered tissues still fall short of native tissues. Significant additional work is clearly required to identify the types of mechanical stimulation required to optimize the formation of mechanically competent engineered tissues. A limitation to date has been the lack of suitable experimental systems that can readily provide a range of relevant mechanical, and possibly magnetic or electrical, stimulation to three-dimensional engineered tissues in sufficient numbers to allow large-scale screening studies to be performed. A new device (Figure 6.5) has recently been developed in the laboratory of Robert Dennis at the University of Michigan that meets these criterion for engineered muscle tissue, and the development of similar systems will be key to accelerating progress with other tissues as well.

SUMMARY

Clearly, a large number of design aspects must be considered to engineer tissues for clinical applications. There has been considerable work recently in many of these areas, with promising results. However, significant work remains in each of these areas. Table 6.1 provides an estimation of both the current knowledge base in each of the areas discussed in this chapter, as well as an indication of the amount of work done to date in each area.

It is important to recognize that these design issues do not exist in isolation, but there is significant synergy among these variables in some situations. For example, the biomaterials and biomechanics design issues may need to be considered together. It has recently been demonstrated that engineered smooth muscle tissues only respond to mechanical stimuli and form stronger tissues when adherent to specific types of adhesion molecules on the scaffolds (Kim et al. 1999). Similarly, mass transfer issues may have significant impact on the mechanical properties of engineered tissues, as recently described for cartilage grown in vitro (Vunjak-Novakovic et al. 1999). A variety of other interactions will likely emerge as this field is further developed.

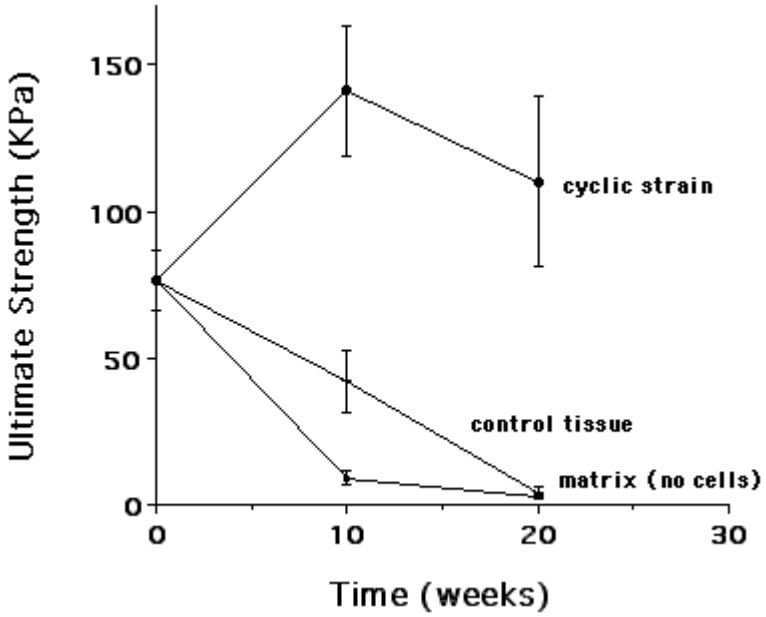


Fig. 6.4. Representation of the ultimate strength of engineered smooth muscle tissues subjected to mechanical stimulation (cyclic strain), no strain (control tissue), and the scaffolds alone (matrix (no cells)) over time in culture. Tissues were engineered with Type I collagen sponges and were maintained in culture for the indicated time periods in serum containing medium. Cyclic strain consisted of 7% amplitude strain at 1 Hz. (Adapted from Kim et al. 1999.)

Fig. 6.5. Novel device for applying specific regimens of mechanical and/or electrical stimulation to engineered tissues *in vitro* developed in the laboratory of R. Dennis (University of Michigan). *Left*. The system is modular and is designed to operate in stacks of 6 units per tower in an incubator. *Center*. The system uses standard cell-culture disposable plastic dishes. Individual tissue constructs are grown in 35 mm-diameter culture dishes. A 100 mm-diameter culture dish houses the tissue in the 35 mm culture dish, a servo motor, a force transducer, a stepper driver, a high-voltage bipolar stimulator, and two microcontrollers. The units are interchangeable and connect with the main power and data bus via a 25-pin D-sub connector. *Right*. A close up view of the prototype device, showing the force transducer in the foreground in the 100 mm dish, the servomotor in the background, and the electronics module to the left of the 35 mm culture dish. The mounting fixtures for the tissue construct and the electrodes are not shown. Micropower techniques have been employed to minimize power dissipation and heat accumulation (R. Dennis; used by permission).

Table 6.1
Current Levels of Knowledge and Research
in the Engineering Design Aspects of Tissue Engineering

	Knowledge base	Work to date
Bioreactors for 2D cell expansion	Extensive	Extensive
Bioreactors for 3D tissue growth	Modest	Modest
Liver and kidney assist bioreactors	Modest	Modest
Promoting vascularization of engineered tissues	Modest	Little
Cell storage technology	Extensive	Extensive
Storage of three-dimensional engineered tissues	Modest	Little
Identifying mechanical properties of native tissues	Modest	Extensive
Identifying the minimum properties required of engineered tissues	Little	Little
Mechanical signals regulating cell function	Extensive	Extensive
Mechanical signals regulating engineered tissues	Little	Little

REFERENCES

Ajioka, I., T. Akaike, and Y. Watanabe. 1999. Expression of vascular endothelial growth factor promotes colonization, vascularization, and growth of transplanted hepatic tissues in the mouse. *Hepatology*. 29 (2):396-402.

Anchordoquy, T.J., and G.S. Koe. 2000. Physical stability of nonviral plasmid-based therapeutics. *Journal of Pharmaceutical Sciences*. 89 (3):289-296.

Ando J., R. Korenaga, and A. Kamiya. 2000. Shear stress-dependent regulation of endothelial cell functions. In K. Suzuki, Y. Ikeda, and I. Maruyama, Eds. New frontier in vascular biology: Thrombosis and hemostasis. Eibun Press, Ltd. (Osaka, Japan).

Bachoud-Levi, A.C., N. Deglon, J.P. Nguyen J. Bloch, C. Bourdet, L. Winkel, P. Remy, M. Goddard, J.P. Lefaucheur, P. Brugieres, S. Baudic, P. Cesaro, M. Peschanski, and P. Aebischer. 2000. Neuroprotective gene therapy for Huntington's disease using a polymer encapsulated BHK cell line engineered to secrete human CNTF. *Human Gene Therapy*. 11 (12):1723-9.

Bischof, J.C. 2000. Quantitative measurement and prediction of biophysical response during freezing in tissues. *Annual Review of Biomedical Engineering.* 2:257-288.

Butler, D.L., S.A. Goldstein, and F.Guilak. 2000. Functional tissue engineering: the role of biomechanics. *Journal of Biomechanical Engineering*. 122(6):570-5, Dec.

Carmeliet, P, and R.K. Jain. 2000. Angiogenesis in cancer and other diseases. *Nature* 407:249-257.

Cao, Y., J.P. Vacanti, X. Ma, K.T. Paige, J. Upton, Z. Chowanski, B. Schloo, R. Langer, and C.A. Vacanti. 1994. Generation of neo-tendon using synthetic polymers seeded with tenocytes. *Transplant. Proc*. 26:3390-3392.

Carver, S.E., and C.A. Heath. 1999. Increasing extracellular matrix production in regenerating cartilage with intermittent physiological pressure. *Biotechnology & Bioengineering.* 62 (2):166-74.

Colton, C.K. 1995. Implantable bioartificial organs. *Cell Transplant*. 4:415-436.

_____. 2000. Mass transfer issues in tissue engineering. In *WTEC Workshop on Tissue Engineering Research in the United States, Proceedings, June 5, 2000*. Baltimore, MD: International Technology Research Institute, Loyola College.

Dennis, R.G., and P.E. Kosnik. 2000. Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered in vitro. *In Vitro Cellular & Developmental Biology. Animal* 36 (5):327-335.

Emerson, S,G., B.O. Palsson,. and M.F. Clarke. 1991. The construction of high efficiency human bone marrow tissue ex vivo. *Journal of Cellular Biochemistry* 45(3):268-72.

Fang J., Y.Y. Zhu, E. Smiley, J. Bonadio, J.P. Rouleau, S.A. Goldstein, L.K. McCauley, B.L. Davidson, and B.J. Roessler. 1996. Stimulation of new bone formation by direct transfer of osteogenic plasmid genes. *Proceedings of the National Academy of Sciences of the United States of America*. 93 (12):5753-8.

Giancotti, F.G., and E. Ruoslahti. 1999. Integrin signaling. *Science* 285:1028-1032.

Guilak, F. 2000. Functional tissue engineering of articular cartilage: The role of biomechanics. In *WTEC Workshop on Tissue Engineering Research in the United States, Proceedings, June 5, 2000*. Baltimore, MD: International Technology Research Institute, Loyola College.

Ingber, D.E. 1991. Integrins as mechanochemical transducers. *Curr. Opin. Cell Biol*. 3:841-848.

Karlsson, J.O.M., and M. Toner. 2000. Cryopreservation. In R.P. Lanza et al. (ed.) *Principles of Tissue Engineering* (2nd ed.), Academic Press.

Kim, B.S., J. Nikolovski, J. Bonadio, and D.J. Mooney. 1999. Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nature Biotechnology.* 17 (10):979-983.

Konstantopoulos K., and L.V. McIntire. 1997. Effects of fluid dynamic forces on vascular cell adhesion. (Review; 16 refs.) *Journal of Clinical Investigation* 100 (11 Suppl):S19-23.

Lysaght, M.J.,. B. Frydel, F. Gentile, D. Emerich, and S. Winn. 1994. Recent progress in immunoisolated cell therapy. [Review; 48 refs.] *Journal of Cellular Biochemistry* 56 (2):196-203.

Mauck, R.L., M.A. Soltz, C.C.B. Wang, D.D. Wong, P.H.G. Chao, W.B. Valhmu, C.T. Hung, and G.A. Ateshian. 2000. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J. Biomech. Eng*. 122:252-260.

McLaughlin, B.E., C.M. Tosone, L.M. Custer, and C. Mullon. 1999. Overview of extracorporeal liver support systems and clinical results. *Annals of the New York Academy of Sciences* 875:310-25.

Mikos, A.G., G. Sarakinos, M. Lyman, D.E. Ingber, J. Vacanti, and R. Langer. 1993. Prevascularization of porous biodegradable polymers. *Biotech. Bioeng*. 42:716-723.

Miller, W.M. 2000. Bioreactor design considerations for cell therapies and tissue engineering. In *WTEC Workshop on Tissue Engineering Research in the United States, Proceedings, June 5, 2000*. Baltimore, MD: International Technology Research Institute, Loyola College.

Nakamura, N., D.A. Hart, R.S. Boorman, Y. Kaneda, N.G. Shrive, L.L. Marchuk, K. Shino, F. Ochi, and C.B. Frank. 2000. Decorin antisense gene therapy improves functional healing of early rabbit ligament scar with enhanced collagen fibrillogenesis in vivo. *Journal of Orthopaedic Research* 18 (4):517-523.

Naughton, G. 1999. The advanced tissue sciences story. *Scientific American* 280 (4):84-5.

Nerem, R.M. 1993. Hemodynamics and the vascular endothelium. *J. Biomech. Eng*. 115:510-514.

Niklason, L.E., J. Gao, W.M. Abbot, K.K. Hirschi, S. Houser, R. Marini, and R. Langer. 1999. Functional arteries grown in vitro. *Science* 284:489-493.

Nikolovski, J., E. Gulari, and H.D. Humes. 1999. Design engineering of a bioartificial renal tubule cell therapy device. *Cell Transplantation* 8 (4):351-64.

Nor, J.E., J. Christensen, D.J. Mooney, and P.J. Polverini. VEGF enhances the survival of endothelial cells and sustains angiogenesis by inducing expression of Bcl-2 *Am. J. Pathol.* 154:375-384.

Obradovic, B., R.L. Carrier, G. Vunjak-Novakovic, and L.E. Freed. 1999. Gas exchange is essential for bioreactor cultivation of tissue engineered cartilage. *Biotechnology & Bioengineering* 63 (2):197-205.

Ochiya, T., Y. Takahama, S. Nagahara, Y. Sumita, A. Hisada, H. Itoh, Y. Nagai, and M. Terada. 2000. New delivery system for plasmid DNA using atecollagen as a carrier material: The minipellet. *Nature Medicine* 5:707-710.

Parenteau, N. 1999. Skin: The first tissue-engineered products. *Scientific American* 280 (4):83-4.

Seliktar, D., R.A. Black, R.P. Vito, and R.M. Nerem. 2000. Dynamic mechanical conditioning of collagen-gel blood vessel constructs induces remodeling in vitro. *Annals of Biomedical Engineering* 28 (4):351-362.

Shea, L.D., E. Smiley, J. Bonadio, and D.J. Mooney. 1999. DNA delivery from polymer matrices for tissue engineering. *Nature Biotechnology* 17 (6):551-554.

Sheridan, M.H., L.D. Shea, M.C. Peters, and D.J. Mooney. 2000. Bioadsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. *Journal of Controlled Release* 64 (1-3):91-102.

Shyy, J.Y.J., and S. Chien 1997. Role of integrins in cellular responses to mechanical stress and adhesion. *Curr. Opin. Cell Biol*. 9:707-713.

Sun, Y., X. Ma, D. Zhou, I. Vacek, and A.M. Sun. 1996. Normalization of diabetes in spontaneously diabetic cynomologus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. *J. Clin. Invest*. 98:1417-1422.

Tabata,Y. 2000. The importance of drug delivery systems in tissue engineering. *Pharm. Sci. Technol. Today* 3 (3): 80-89.

Vandenburgh, H.H., S. Hatfaludy, P. Karlisch, and J. Shansky. Mechanically induced alterations in cultured skeletal muscle growth. *Journal of Biomechanics.* 24 Suppl 1:91-9.

Vunjak-Novakovic, G., I. Martin, B. Obradovic, S. Treppo, A.J. Grodzinsky, R. Langer, and L.E. Freed. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *Journal of Orthopaedic Research* 17 (1):130-8.

Wang, W. 2000. Lyophilization and development of solid protein pharmaceuticals *International Journal of Pharmaceutics*. 203 (1-2):1-60.

CHAPTER 7

INFORMATICS AND TISSUE ENGINEERING

Peter C. Johnson

INTRODUCTION

Informatics as applied to tissue engineering is perhaps the most futuristic of the topic areas covered in this WTEC study. While this chapter will review the activities detected worldwide in the course of the study, it will also define a template for the future development of this aspect of the field of tissue engineering.

Informatics is actually a descriptive term used in reference to the application of information science tools especially to health care and related research. The *Merriam Webster Collegiate Dictionary* (10th ed.) defines information science as "the collection, classification, storage, retrieval, and dissemination of recorded knowledge treated both as a pure and as an applied science." Informatics as a concept applied to biological research has two key components: "bioinformatics" and "computational biology." The National Institutes of Health (NIH) Biomedical Information Science and Technology Initiative Consortium has defined these as follows (http://grants.nih.gov/grants/bistic/CompuBioDef.pdf):

> *Bioinformatics:* Research, development, or application of computational tools and approaches for expanding the use of biological, medical, behavioral or health data, including those to acquire, store, organize, archive, analyze, or visualize such data.
>
> *Computational Biology:* The development and application of data-analytical and theoretical methods, mathematical modeling, and computational simulation techniques to the study of biological, behavioral, and social systems.

Thus deployed, the terminology of informatics refers to the capacity to digitally capture, manage, extract value from, and rapidly share the complexity of scientific discoveries. Informatics has been driven by the availability of computer systems, the Internet, and the massive increase in scientific data over the past several decades. Its application to the analysis of genetic, protein, cellular, and health care information is quite mature. Its application to tissue information in general and tissue engineering in particular is less well developed. However, it is clear that informatics will play an increasingly large role in tissue engineering for the following three reasons:

1. In order to properly design and characterize engineered tissues, it will inevitably be necessary to apply tools and information from all other areas of informatics (for example, genomics and proteomics) in a more routine fashion.
2. Information that describes tissues themselves both structurally and functionally will require massive storage and analysis capabilities.
3. The international population of tissue engineers will need to leverage digital communication mechanisms to collaborate on, learn, and harmonize both standards and regulatory practices as tissue engineering comes to serve worldwide markets.

A useful way in which to depict the emergence of informatics is to review what is known as the Continuum of Bioinformatics (Figure 7.1). As illustrated, the human being can be described by a series of scaled data sets that include molecules, cells, tissues, and ultimately, the whole human. Not shown, though very important to biomedical research, are the additional categories of organismal behavior (especially in health and disease) and the behaviors of societal groups. To obtain the information descriptive of each level of scale, raw data access technologies are applied. For example, DNA sequencing/expression microarrays and MALDI-TOF mass spectrometry are used to obtain genomic and proteomic data, respectively. Cell information is obtained through imaging and biochemical assays. Tissue information can be acquired using histopathology and automated machine vision analysis, including functional analysis in the presence of probes such as *in situ* hybridization to detect mRNA and immunohistochemistry to detect proteins. This type of information-gathering is highly dependent upon multiple modalities of microscopic imaging that are collectively known as mesoscopic imaging. Whole organism information can be obtained using MRI scans, CT scans, and photography — and especially, as in the case of the Visible Human Project, using a combination of the three.

As this data is acquired, the potential exists to knit the data together into computing models that acceptably reflect the complexity of the processes occurring at each level of biological complexity. One overall model of this type is known as the Physiome. Considerable data acquisition will be required before the Physiome concept can be put into full practice. This quantity of data, generated by multiple mechanisms and prepared for cross-correlation of the different scales, requires substantial computing power and organization. Enter informatics, the great enabler of this process.

What has been the driving force for aggregating this data? While federal monies have been invested in informatics heavily in the United States for the past ten years, informatics has had its major genesis in the support

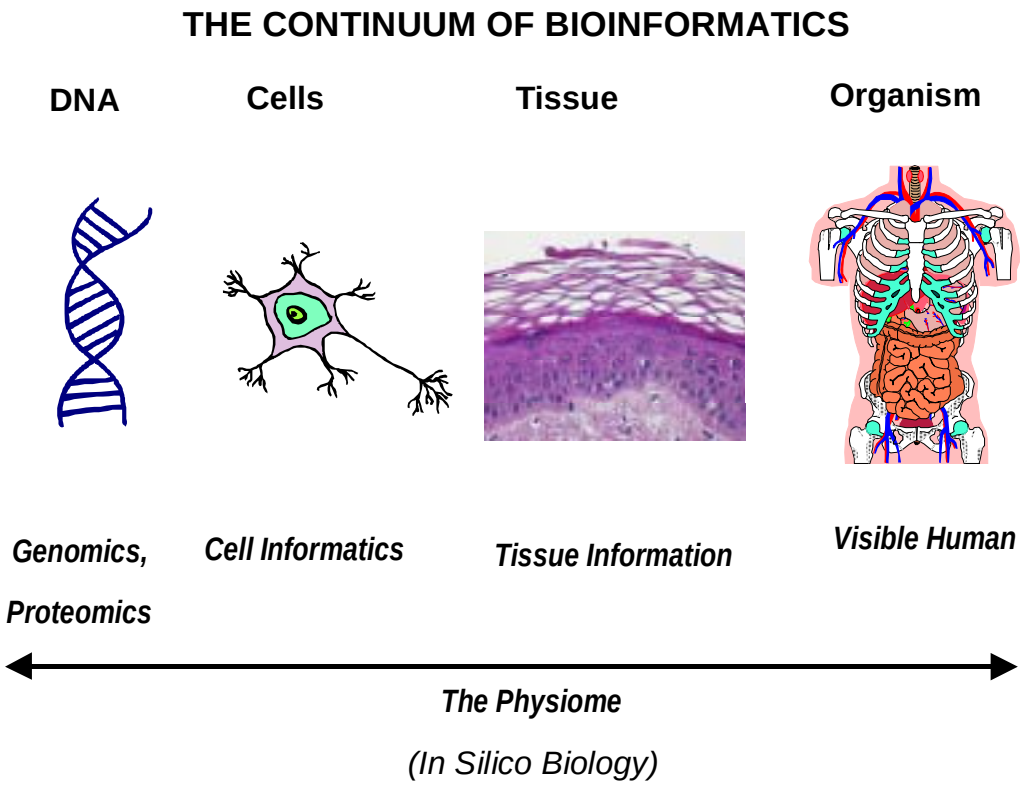


Fig. 7.1. Bioinformatics deals with discrete sets of information (such as the sequence of the human genome) and with the correlation of data between sets of information (such as between the presence of active genes and the cell, and between tissue and whole person manifestations of that gene activity). The breadth of bioinformatics demands novel solutions to the management of very different but related data. The power of bioinformatics lies in its ability to generate rapid association between cause and effect across the entire continuum.

of pharmaceutical drug discovery. This process requires that large numbers of related molecular, cellular, tissue, and clinical events be cross-correlated to enable identification of novel drug targets (typically proteins) by eliciting their differential expression in diseased and normal tissues. In addition, computing power has been needed to provide modeling support for the three-dimensional structure of proteins and drugs. In a world where a single added day of patent life can be worth millions of dollars in revenues, informatics has provided the pharmaceutical industry with the potential to remove randomness from the drug discovery process and capture knowledge digitally to accelerate the time to market of new drugs. Moreover, the underlying value of informatics as a perpetuator of access to information is also at play. In the year 2000, $4 billion were spent on informatics solutions by the pharmaceutical industry. By 2003, this is expected to grow to $9 billion (PhRMA 2000). It is predicted that the tissue engineering industry will benefit from mature informatics technologies that in turn will help accelerate the development of the tissue engineering industry as well.

A second major genesis of bioinformatics has been the push for the sequencing of the human genome, which created data volume and logistical considerations that could only be managed via advanced computing methods. A final driver has been the constellation of health care systems that needed to respond with data to the inquiries of insurers, the federal government, and doctors and patients. This led to the onset of formal health care informatics processes—a key component of the continuum of bioinformatics once descriptive data from the human organism itself is aggregated. With these combined data sets, sense can be made of the role of genes in disease, tissue responses to genes, and so on.

Of all of the informatics sectors, genomics is the best developed, as illustrated in the following partial list of the world's most prominent bioinformatics centers (Table 7.1). Note that such centers are now being established throughout the world.

Table 7.1
Major Bioinformatics Centers

Center	Location	Prime Focus	Web Site
DDBJ (DNA Data Bank of Japan)	Japan	Genomics/ Proteomics	http://www.ddbj.nig.ac.jp/
EMBL (European Molecular Biology Laboratory)	Europe (Heidelberg, Germany)	Genomics/ Proteomics	http://www.embl-heidelberg.de/
EBI (European Bioinformatics Institute)	Europe (Cambridge, UK)	Genomics/ Proteomics	http://www.embl-heidelberg.de/
ICCB (International Center for Cooperation in Bioinformatics)	Israel (Weizmann Institute)	Genomics/ Proteomics	http://www.iccbnet.org/overview.html
NCBI (National Center for Biotechnology Information)	U.S. (Bethesda, MD)	Genomics/ Proteomics	http://www.ncbi.nlm.nih.gov/
Stanford Human Genome Center	U.S. (Palo Alto, CA)	Genomics	http://shgc-www.stanford.edu/
TIGR (The Institute for Genomic Research)	U.S.	Genomics	http://www.tigr.org/
The Sanger Centre	Europe (Cambridge, UK)	Genomics	http://www.sanger.ac.uk/
SWISS-PROT	Europe (Switzerland)	Proteomics	http://us.expasy.org/sprot/
UK Human Genome Mapping Project Resource Centre	UK	Genomics	http://www.hgmp.mrc.ac.uk/
Washington University, St. Louis	U.S.	Genomics	http://www.genetics.wustl.edu/
Weizmann Institute of Science	Israel	Genomics/ Proteomics	http://bioinformatics.weizmann.ac.il/
Whitehead Institute	U.S. (Cambridge, MA)	Genomics	http://www.wi.mit.edu/

INFORMATICS COMPONENTS THAT WILL ULTIMATELY SUPPORT TISSUE ENGINEERING

Today, an Internet/PubMed search using the terms “tissue engineering” and “bioinformatics” prompts essentially no responses; these fields are in their infancy. Until now, tissue engineering has been primarily empirical (“Edisonian”) in nature, with investigators relying on cell-directed behavior, often within matrices, to direct the ultimate structures and functions of engineered tissues. These tissues have been restricted in their complexity by a technical inability to generate three-dimensional vascular networks; therefore, tissue engineering has been restricted to the use of thin, essentially two-dimensional tissues such as skin and cartilage. These tissues have not yet been of sufficient complexity to demand informatics-based design approaches.

As an extension of traditional practice, pathologists have provided pattern recognition and matching at the histological level for manufacturers of engineered tissues. They also have provided post-manufacture quality assurance services for those companies that manufacture and sell engineered tissues. The regulatory processes thus far have not required extensive use of functional testing at the genetic or proteomic level; therefore, tissue engineering has been late to be affected by the field of bioinformatics.

This is changing. As scientific and regulatory processes grow in their complexity — and as we CAN know more about the tissues we are creating — it is likely that engineered tissues will need to be characterized genetically and in other functional ways that will demand access by tissue engineers to all of the tools of genomics and proteomics. In addition, the emergence of cellular informatics and machine vision tools to quantitatively characterize every aspect of tissue structure (and many aspects of tissue function) inevitably will require data storage and retrieval systems as well as data mining functionality.

Ultimately, as more and more cellular and tissue information pools are acquired, a process similar to that seen in genomics is likely to emerge. First, companies will develop subsets of information for sale or application. Next, government funding will enable the creation of vast data troves related to tissues. Finally, public companies will identify niche opportunities for data mining and data application (such as *in silico* modeling and CAD/CAM manufacture of tissues) that will further drive the need for informatics tools in tissue engineering.

The following table lists several of the functionalities that will be needed to support the penetration of informatics into tissue engineering. In most cases, these technologies are already in place. Only in a few instances are they rate-limiting, and these instances have been annotated as such in Table 7.2.

Table 7.2
Functionalities Needed To Drive Informatics In Tissue Engineering

Functionality	Role and Rationale
Computing backbone (servers, Internet, Internet II, supercomputing)	
Gene and protein sequencing	
Gene expression analysis	
Protein expression and interaction analysis	Rate limiting—complexity issues
Quantitative cellular image analysis	
Quantitative tissue analysis	Rate limiting—technology in development
In silico modeling	Rate limiting—awaiting data
Digital tissue manufacturing	Rate Limiting—awaiting data and technologies
Digital quality assurance systems	
Data mining tools	
Clinical informatics interface	

Of great background importance are the supercomputing centers and activities being developed throughout the world. (For a comprehensive listing of supercomputing centers worldwide see the web site http://parallel.rz.uni-mannheim.de/docs/ind.html.) Ultimately, these will become more and more important

in the provision of services to the bioinformatics community, since they will be able to provide both storage and processing speed at the proper levels for data-intensive investigations. Two commercial enterprises bear watching: Blue Gene, the supercomputer being developed by IBM for bioinformatics, and the Celera-Compaq-Sandia labs alliance for supercomputing development in bioinformatics.

Table 7.3 describes the degree of maturity of informatics components in development worldwide. As data-generation techniques are applied, several types of "products" will be generated that will be of service to the entire tissue-engineering enterprise. They are included in Table 7.4, with their rationales.

Table 7.3
Maturity of Informatics Components Worldwide

Informatics Component	Clarifying Example	Level of Maturity*
Genome sequencing	Nucleotide sequencing and mapping paradigms	Mature
Genome function analysis (genomics)	mRNA expression in tissues, single nucleotide polymorphism analysis	Emerging
Protein sequencing	Amino acid sequencing	Mature
Protein function analysis (proteomics)	3-D protein shape, expression within tissues, protein to protein interactions	Nascent
Cellular information capture tools	Imaging and probes, especially fluorescent	Emerging
Cellular information analysis	Databases containing pathway-specific cellular response data	Nascent
Tissue information capture tools	Machine vision and tissue-specific automated software	Nascent
Tissue information analysis	Content based pattern retrieval and mathematical characterization of tissues	Nascent
Whole organism information capture tools	MRI, CT and other imaging modalities	Mature
Whole organism information analysis	Software to automate the analysis of clinical images	Emerging
Healthcare information systems	Patient care data, outcomes data	Emerging
In silico modeling	Computational depiction of cellular pathways, interacting as in life and structure of cells and tissues to provide virtual organ environments	Nascent
Supercomputing	Data storage and management	Mature

*The scale used to define maturity (nascent, emerging, and mature) is subjective. Note that the tissue information components that relate most directly to tissue engineering are at the earliest stages of development.

U.S. R&D ACTIVITIES

The United States is in a leading position in every sector of bioinformatics at this time. The Human Genome Project, the Physiome Project, and the new NIH Center for Bioengineering and Imaging are but a few focused mechanisms that leverage the enormous NIH and other federal scientific budgets to move this field forward. Moreover, the United States also leads in the commercialization of the technologies that emerge all across the continuum of bioinformatics, with substantial companies emerging at this time in each sector, as shown in Table 7.5. The focus of this chapter will therefore be on the efforts underway in Europe and Japan.

Table 7.4
Data Products of Relevance to Tissue

"Product"	Rationale
Tissue structural databases	Provide a sampling basis for rational tissue design
Tissue functional databases	Provide grouped data for assessment of engineered tissue functional responses
Tissue biomaterial response databases	Provide a characterization of normal versus engineered tissues in their responses to implants
Tissue data analysis tools	Data mining for unique tissue structural and functional relationships that will affect assessment of efficacy and safety
Standardized manufacturing datasets	To simplify and make repetitive the manufacturing process
Tissue modeling systems -Scaffold Design and Manufacture -Cellular placement technologies	To envision and design tissues (Example) (Example)
Automated quality-assurance systems	To assess engineered tissues for lot-to-lot variability so as to ensure conformance with regulatory guidelines

Table 7.5
Table of Representative Companies in the Continuum of Bioinformatics Companies (Sample)

Bioinformatics Focus	Representative Companies
Genomics	Celera, Human Genome Sciences, Genomics Collaborative, GeneLogic
Proteomics	Axcell, Ciphergen; Cambridge Antibody Technology (UK)
Cell information	Cellomics; Aurora
Tissue information	TissueInformatics; Resolution Sciences; Chromavision
Whole human	All CT/MRI Imaging Companies
Physiome	Physiome Sciences
Data mining and visualization	Informax; IBM, Spotfire; Silicon Genomics

EUROPEAN R&D ACTIVITIES

Europe has demonstrated significant interest in the development of capability in bioinformatics, as shown above in Table 7.1. With respect to tissue engineering, there is modest development occurring in the informatics arena. The following lists European R&D centers and activities in the imaging, modeling, genomics, and related technology sectors.

Imaging, Archiving, Modeling, and Related Technologies

- *Berlin Charite/German Rheumatism Center*. Digital imaging, archiving, and local networking of research images are prepared for shared access by researchers on a common system.
- *European Molecular Biology Laboratory (EMBL) (Heidelberg).* Optical and laser tweezers have been developed for fabrication of cellular structures. These are not yet guided completely by informatics systems. Notably, the EMBL was the site of development of the BioImage Database (www.bioimage.org/functional), an attempt to aggregate biological images with all associated information from research projects. This has stalled due to funding problems. The EMBL also has strong imaging capabilities, especially in the areas of confocal imaging and image processing.
- *German Cancer Research Center (Heidelberg).* Using the *c. Elegans* development model, this center is performing 3D and 4D imaging and is developing image databases, fully automated dynamic imaging tools, and data mining and modeling methods, which may be particularly applicable to tissue engineering.

- *German Heart Institute (Berlin).* CT-based informatics is being applied to rapid prototyping of pulmonic heart valves. This is a direct example of the application of informatics systems to rational tissue design. Virtis, a for-profit subsidiary, is being formed to commercialize this technology.
- *Kirchoff Institute for Physics (Heidelberg).* Confocal imaging and image processing have been developed to track nuclear parameters in cells.
- *MeVis Center for Medical Diagnostics Systems and Visualization, University of Bremen*. The MeVis Center has both nonprofit and for-profit components. Its competencies are imaging, data compression technology, remote imaging, shared "networks of competence," and 2D and 3D image analysis and wavelet analysis. This center is well positioned to add value in image analysis aspects of tissue data acquisition.

Data-Based Modeling/Placement Technologies

- *ETH (Zurich).* Optical waveguides have been developed to place molecules in two and three dimensions for cytoskeletal modeling. This is a higher resolution form of emerging technologies that use informatics control mechanisms to rationally guide placement of cells and matrix in three dimensions.
- *Liverpool Biocomputation Group, the Computational Liver*. Though not visited by the WTEC team, this group's site (somewhat dated) can be found on the Internet. This is an example of organ-based informatics, a component of the Physiome (http://www.csc.liv.ac.uk/~biocomp/research/cells_tissues.html).
- *Imperial College of Science, Technology and Medicine/Chemical Engineering and Chemical Technology Dept. (London).* Digital imaging is being performed for 3D modeling and manufacture of bone marrow for hematopoiesis assessment—an example of rational tissue design and manufacture using imaging and informatics tools.
- *University of Glasgow*. 2-D neural patterning is being done in association with the Max Planck Institute and a microelectronics group. Here, tissue understanding is being converted through technology into a fabrication environment whose success can be quantitatively determined using imaging and informatics on the outflow side.

Genomics and Proteomics

- *INSERM (Bordeaux).* A strong emphasis is now being placed on functional genomics.
- *Imperial College of Medicine, Orthopedic Surgery Department (London).* Microarray technology is being employed for the determination of differentiation of osteogenic precursors. Microarray technology, in particular, requires strong informatics support systems because each "experiment" can generate upwards of 10,000 data points.
- *Charing Cross Hospital (London).* Correlative mechanical assessment of cartilage is being performed using proteomics approaches, an example of the continuum of bioinformatics being bridged between the molecular and the tissue scale.
- *EMBL/European Bioinformatics Institute (Heidelberg).* This institute has a strong and emerging genomics and proteomics focus with associated informatics components.

In summary, Europe is demonstrating strengths in genomics, proteomics, imaging, and fabrication technologies that will position it well for coming developments in bioinformatics applications to tissue engineering.

JAPANESE R&D ACTIVITIES

Japanese activities are emerging in the informatics sector as applied to tissue engineering. The following interesting examples apply:

- *Hokkaido University*. Here 3D scaffold assembly is being performed, although with minimal, if any, informatics support.
- *Keio University*. Perhaps the most vibrant example of applied informatics in Japan is the e-Cell Initiative at Keio University. Much like the Physiome Project in the United States, its goal is to map the

networks of gene products and pathways in the living cell to predict responses to drugs and other perturbations. This is an example of *in silico* biology at the cellular level—one which will eventually be extended to models of multicellular organs, groups of organs, and eventually, whole organisms. The e-Cell Initiative has a web site, http://www.e-cell.org.

- *National Cancer Center Research Institute (NCCRI) (Tokyo).* A newly wrought Cancer Genomics Project is underway at this institute.
- *National Institute of Bioscience and Human Technology (Tsukuba).* The following three centers were to be established at this institute in 2001:
  (a) Computational Biology Research Center
  (b) Structural and Functional Genomics Research Center
  (c) Gene Discovery Research Center

  This represents a substantial Japanese investment in applied bioinformatics and genomics. This institute is likely to become a dominant center of tissue-engineering-related informatics R&D activity.
- *Institute of Physical and Chemical Research (RIKEN) (Tsukuba).* The Genomics Center here is a mirror site for the Human Genome Initiative.
- *Tokyo Women's Medical University (Tokyo).* Photolithography technology is being developed here for polymer deposition. This is an example of an informatics-driven manufacturing technology.

The Japanese government is beginning to focus resources on the fields of bioinformatics and tissue engineering at significant levels. Japan's emergence in these sectors in the next 2-5 years should provide it with competitive advantages in niche sectors of the bioinformatics continuum and especially, perhaps, in fabrication technology.

SUMMARY

In summary, informatics as applied to tissue engineering draws on a strong but recent heritage of its application to pharmaceutical drug discovery processes, molecular biology research, and management of health care information. New tools such as machine vision/automated tissue analysis software, cellular functional probes, and databases whose standard construction enables cross-talk will soon find application in the design, development, and characterization of engineered tissues. The nature of the Internet will enable scientists worldwide to gain access to data that is relevant to this process, as well as to one another.

At this time, the United States is the world leader in informatics applications to biological research, not only because of its strong pharmaceutical industry "pull" on bioinformatics but also because personal computing, supercomputing, database development, and Internet-based data transfer have generally been U.S. strengths. Delays in investment in this sector in Japan and social issues that delayed biotechnology development in Europe were factors in their lag. However, strong institutional developments are occurring in both Japan and Europe that will make collaborations possible and *de novo* technology development competitive.

What Does the Future Hold?

It seems clear from reviewing related areas of research that informatics platforms will accelerate all research in tissue engineering, not only by providing scientists access to critical information but also by providing more scientists access to one another. The development of tissue information creation and analysis technologies for the support of pharmaceutical drug discovery will have a spin-off benefit in tissue engineering, by setting standards for the further development of such tools as applied to the creation of engineered tissues. No doubt as genetic analysis capabilities continue to mature, engineered tissues will be subjected to informatics-type analyses—especially given the advent of complete tissue genetic characterization using microarrays. Such data will require handling and information systems similar to those presently used in the pharmaceutical industry. The need to analyze tissue information per se in tissue engineering may also prove to be a synergistic driver, moving this information into the purview of pharmaceutical drug discovery sooner than it might otherwise have been.

With substantial databases of tissue structure and function in hand—and this is certainly an area in which federal support should be considered—rational tissue engineering design in three dimensions can be contemplated. If properly culled, such data can provide the tissue component location coordinates to support CAD/CAM tissue manufacture and automated QA systems to ensure minimal lot-to-lot variability between tissues engineered to meet standards. The creation of standards for the development of tissue-engineered medical products has been in process for the past four years through the American Society for Testing and Materials (Committee F04, Division IV on Tissue Engineered Medical Products). The continued development of such standards for the management and application of tissue, cellular, and molecular information will increasingly make the sharing of common technologies smoother. The growing international role of this organization in standards activities may well set the stage for shared standards for informatics in tissue engineering that can foster worldwide collaboration and accelerate the development of complementary technologies for this important sector in health care research.

The following table illustrates the state of progress in the United States, Europe, and Japan at the present time. It is likely that growth will occur in all areas in all three geographic sectors in the same relative scales for the near future, since all are investing heavily in informatics at this time.

Table 7.6
State of Progress in the United States, Europe, and Japan

Activity	Knowledge Base	Work to Date	Leading Region
Genomics	Advanced	Extensive	U.S.>UK>Switzerland
Proteomics	Incomplete	Significant	U.S.
Microarray	Advanced	Extensive	U.S.
Cell Information	Incomplete	Significant	U.S.
Tissue Information	Little	Little	U.S., Germany
Physiome (System)	Incomplete	Significant	U.S.>Japan
Commercial	Incomplete	Significant	U.S.>Germany

REFERENCES

The following is a bibliography of selected articles to familiarize the reader with the emerging zones of informatics and informatics applications that will affect tissue engineering

Ackerman, M.J. 1991. The visible human project. *J. Biocommun* 18 (2):14.

Altman, R.B. 2000. The interactions between clinical informatics and bioinformatics: A case study. *J Am Med Inform. Assoc.* 7 (5) (Sep-Oct):439-43.

Bassingthwaighte, J.B. 2000. Strategies for the physiome project. Review. *Ann. Biomed. Eng.* 28 (8) (Aug):1043-58.

Becich, M.J. 2000. The role of the pathologist as tissue refiner and data miner: The impact of functional genomics on the modern pathology laboratory and the critical roles of pathology informatics and bioinformatics. *Mol. Diagn.* 5 (4) (Dec.):287-99.

Benson, D.A., I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, B.A. Rapp, and D.L. Wheeler. 2000. GenBank. *Nucleic Acids Res.* 28 (1) (Jan 1):15-8.

Cheng, E.J., I.E. Brown, and G.E. Loeb. 2000. Virtual muscle: A computational approach to understanding the effects of muscle properties on motor control. *J. Neurosci Methods* 101 (2) (Sep 15):117-30.

Fiechter, A. 2000. Biotechnology in Switzerland and a glance at Germany. Review. *Adv. Biochem. Eng. Biotechnol.* 69:175-208.

Greller, L.D., and F.L. Tobin. 1999. Detecting selective expression of genes and proteins. *Genome Res.* 9 (3) (Mar.):282-96.

Pharmaceutical Research and Manufacturers of America (PhRMA). 2000. *PhRMA Industry Profile.* Washington, DC: http://www.phrma.org/publications/publications/profile00/.

Roberts, R. 2000. Bioinformatics analysis of gene banks provides a treasure trove for the functional genomist. *J. Mol. Cell Cardiol.* 32 (11) (Nov.):1917-9.

Roos, D.S. 2001. Computational biology. Bioinformatics—trying to swim in a sea of data. *Science* 291 (5507)(Feb 16): 1260-1.

Stoesser, G., W. Baker, A. van den Broek, E. Camon, M. Garcia-Pastor, C. Kanz, T. Kulikova, V. Lombard, R. Lopez, H. Parkinson, N. Redaschi, P. Sterk, P. Stoehr, and M.A. Tuli. 2001. The EMBL nucleotide sequence database. *Nucleic Acids Res.* 29 (1) (Jan 1): 17-21.

Venter, J.C., M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G Sutton, H.O. Smith, M. Yandell, C.A. Evans, R.A. Holt, J.D. Gocayne, P. Amanatides, R.M. Ballew, D.H. Huson, J.R. Wortman, Q. Zhang, C.D. Kodira, X.H. Zheng, L. Chen, M. Skupski, G. Subramanian, P.D. Thomas, J. Zhang, G.L. Gabor Miklos, C. Nelson, S. Broder, A.G. Clark, J. Nadeau, V.A. McKusick, N. Zinder, A.J. Levine, R.J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I/ Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley, K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, V. Di Francesco, P. Dunn, K. Eilbeck, C. Evangelista, A.E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T.J. Heiman, M.E. Higgins, R.R. Ji, Z. Ke, K.A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li Y. Liang, X. Lin, F. Lu, G.V. Merkulov, N. Milshina, H.M. Moore, A.K. Naik, V.A. Narayan, B. Neelam, D. Nusskern, D.B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, A. Wang, A. Wang, X. Wang, J. Wang, M. Wei, R. Wides, C. Xiao, C. Yan, A. Yao, J. Ye, M. Zhan, W. Zhang, H. Zhang, O. Zhao, L. Zheng, F. Zhong, W. Zhong, S. Zhu, S. Zhao, D. Gilbert, S. Baumhueter, G. Spier, C. Carter, A. Cravchik, T. Woodage, F. Ali, H. An, A. Awe, D. Baldwin, H. Baden, M. Barnstead, I. Barrow, K. Beeson, D. Busam, A. Carver, A. Center, M.L. Cheng, L. Curry, S. Danaher, L. Davenport, R. Desilets, S. Dietz, K. Dodson, L. Doup, S. Ferriera, N. Garg, A. Gluecksmann, B. Hart, J. Haynes, C. Haynes, C. Heiner, S. Hladun, D. Hostin, J. Houck, T. Howland T, C. Ibegwam, J. Johnson, F. Kalush, L. Kline, S. Koduru, A. Love, F. Mann, D. May, S. McCawley, T. McIntosh, I. McMullen, M. Moy, L. Moy, B. Murphy, K. Nelson, C. Pfannkoch, E. Pratts, V. Puri, H. Qureshi, M. Reardon, R. Rodriguez, Y.H. Rogers, D. Romblad, B. Ruhfel, R. Scott, C. Sitter, M. Smallwood, E. Stewart, R. Strong, E. Suh, R. Thomas, N.N. Tint, S. Tse, C. Vech, G. Wang, J. Wetter, S. Williams, M. Williams, S. Windsor, E. Winn-Deen, K. Wolfe, J. Zaveri, K. Zaveri, J.F. Abril, R. Guigo, M.J. Campbell, K.V. Sjolander, B. Karlak, A. Kejariwal, H. Mi, B. Lazareva, T. Hatton, A. Narechania, K. Diemer, A. Muruganujan, N. Guo, S. Sato, V. Bafna, S. Istrail, R. Lippert, R. Schwartz, B. Walenz, S. Yooseph, D. Allen, A. Basu, J. Baxendale, L. Blick, M. Caminha, J. Carnes-Stine, P. Caulk, Y.H. Chiang, M. Coyne, C. Dahlke, A.Mays, M. Dombroski, M. Donnelly, D. Ely, S. Esparham, C. Fosler, H. Gire, S. Glanowski, K. Glasser, A. Glodek, M. Gorokhov, K. Graham, B. Gropman, M. Harris, J. Heil, S. Henderson, J. Hoover, D. Jennings, C. Jordan, J. Jordan, J. Kasha, L. Kagan, C. Kraft, A. Levitsky, M. Lewis, X. Liu, J. Lopez, M. Ma, W. Majoros, J. McDaniel, S. Murphy, M. Newman, T. Nguyen, N. Nguyen, M. Nodell, S. Pan, J. Peck, M. Peterson, W. Rowe, R. Sanders, J. Scott, M. Simpson, T. Smith, A. Sprague, T. Stockwell, R. Turner, E. Venter, M. Wang, M. Wen, D. Wu, M. Wu, A. Xia, A. Zandieh, and X. Zhu., 2000. The sequence of the human genome. *Science*. 291 (5507) (Feb 16):1304-51.

CHAPTER 8

LEGAL AND REGULATORY ISSUES

David Smith

INTRODUCTION

Emerging biomedical products utilizing living tissues present a new order of magnitude of complexity in their interactions with human patients. As such, they challenge established processes for protecting patients and the public health from deleterious adventitious agents, while testing the capacity of those processes to ensure timely access to beneficial therapies. At the same time, using human tissues for purposes of medical product development—or, less benignly, for cloning or optimization of selected functional capabilities—present potentially very troubling legal and ethical issues.

The U.S. Food and Drug Administration (FDA) has been moving toward a comprehensive scheme for the regulation of engineered tissue products over the past eight years, especially since early 1997. FDA's classification and pre-market reviews of first generation engineered tissue products have demonstrated that it is actively engaged in developing rational product approval pathways for engineered tissue products. However, such pathways are available and must function within the limits of a well-established statutory scheme for regulatory classification of medical products, into which engineered tissues do not necessarily fit easily.

This emerging U.S. approach can be contrasted with the present uncertain regulatory status of such products within the European Union and Japan. Inconsistency between regions or a lack of transparency in the application of a national (or, in the case of the EU, pan-national) regulatory authority over engineered tissue products is likely to increase the complexity of introducing new medical technologies incorporating human tissues without materially advancing public health or safety.

While critical to the general advance of medical research, access to human tissues for research or product development is highly sensitive to public disclosure of practices where tissues are taken or used without consent or under circumstances suggesting a commercial market in body parts. The absence of comprehensive federal or state legislation governing "research" tissues deprives the biomedical community of clear, consistent guidelines to follow in acquiring and using tissues, while simultaneously representing a legislative vacuum that may be filled with substantial adverse unintended consequences if done suddenly in response to some public outcry. Absent effective coordination, the initiatives of individual federal agencies to establish policies for research involving human tissues or subjects may impose conflicting requirements or expectations.

FDA REGULATION

Broad authority to control the distribution and sale of medical products in the United States has been granted to the FDA under the federal Food, Drug, and Cosmetic Act (FD&C Act) and the Public Health Service Act (PHS Act). The FD&C Act contains numerous provisions regarding the development and distribution of

medical products, many of which have been introduced or substantially rewritten through a series of amendatory statutes. For example, the 1976 Medical Devices Amendments and 1990 Safe Medical Devices Act significantly expanded and clarified the FDA's authority to regulate medical products classified as devices. Recently, the 1997 FDA Modernization Act (FDAMA) has introduced a number of substantive revisions to a wide range of FDA product approval and enforcement practices; the implications of FDAMA, especially for products derived from emerging biomedical technologies, has yet to be fully realized. The PHS Act contains just two sections of particular importance to FDA regulation of medical products, especially those derived through tissue engineering: §351 prohibits the distribution of unlicensed "biological products" and establishes criteria and procedures the FDA shall observe in issuing such licenses; and §361 empowers the FDA to prevent the spread of communicable diseases

Exercising its authority under these statutes, the FDA has adopted a complex set of regulations that control virtually every aspect of the development and marketing of a medical product according to the potential risk of harm the product may pose to patients or the public health. Thus, the FDA regulates the introduction, manufacture, advertising, labeling, packaging, marketing and distribution of, and record-keeping for, such products. The FDA (also referred to here as the Agency) exercises its regulatory authority over medical products through three divisions, or Centers, each generally responsible for exercising the FDA's regulatory authority over a particular class of medical products, as indicated by their names:

- Center for Drug Evaluation and Research (CDER)
- Center for Devices and Radiological Health (CDRH)
- Center for Biologics Evaluation and Research (CBER)

As a rule, the FDA requires a sponsor of a new medical product to submit a formal application for approval to market the product after the completion of preclinical studies and phased clinical trials that demonstrate to the Agency's satisfaction that the product is safe and effective. The form and review of that request to initiate human trials and the subsequent marketing application vary according to the classification of the product with reference to categories established in the statutes granting regulatory authority to the FDA. In fact, the FDA's classification of a new medical product carries implications beyond identifying the Center responsible for regulatory review or the particular approval pathways the product +may subsequently follow.

Classification of Medical Products

Under current federal law, every medical product is classifiable as a drug, device, biological product (a "biologic"), or "combination product" (that is, a combination device/drug, device/biologic, etc.). The classification of the product determines the particular processes of review and approval the FDA may employ in determining the safety and efficacy of the product for human use.

Under the FD&C Act (at §201(g)(1)), a "**drug**" is broadly defined as:

> . . . [an article] intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease . . . [or] . . . intended to affect the structure or any function of the body.

The FD&C Act (at §201(h)) defines a "**device**" largely by what it is not (that is, neither a drug nor a biologic):

> . . . an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar related article . . .intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease . . . or intended to affect the structure or any function of the body . . . *and which does not achieve any of its primary intended purposes through chemical action within or on the body . . . and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes.* [Emphasis added.]

Finally, the PHS Act (at §351(a)) defines a "**biologic**" as:

> . . . any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product applicable to the prevention, treatment or cure of diseases or injuries.

Not surprisingly, the advance of medical technology has produced products not readily classifiable as drugs, devices, or biologics as those terms have been defined by federal statute. To provide for the expanding varieties of products expressing features of more than one of those classifications, the FDA has been authorized to recognize "combination products." A combination product is classified, assigned to a particular Center, and regulated as a drug, device, or biologic according to its "primary mode of action," as determined by the FDA. Disputes over the classification of a combination product between a sponsor and the FDA or between Centers are submitted to the FDA's Ombudsman for resolution. In fact, the FDA's current approach to the regulation of engineered tissue products began with the Ombudsman's consideration of the classification of the Carticel™ autologous cartilage repair service developed by Genzyme Tissue Repair in 1995.

Implications of Product Classifications

While some medical products simply are what they are (that is, an artificial hip joint is obviously a device and aspirin is clearly a drug), the idea of the combination product suggests that relevant features or intended uses of a new product may exist primarily in the eye of the beholder. At least, the FDA's classification of the product may be influenced by what the sponsor does or does not claim for it and how it has been designed to achieve a particular therapeutic benefit.

Why should the classification of a new medical product for purposes of FDA regulatory review really matter? With few exceptions, all products subject to such review for marketing approval must be safe and effective, regardless of classification. There may be some subtle variation in the measurement of those qualities among the FDA Centers, or approval pathways may seem more efficient or predictable for, say, devices compared to biologics. The real significance of classification lies in the benefits or encumbrances that attach to the product either before or after the actual process of marketing review.

With respect to engineered tissue products, the consequences inuring to the *device* and *biologic* classifications deserve particular attention. First, and most importantly, a medical product cannot be a *device* if its therapeutic or diagnostic benefit is obtained through metabolization, a limitation in the statutory definition of a device that might appear to exclude any product incorporating and depending on the function of any living human tissues. Nevertheless, allogeneic skin products such as Organogenesis's *Appligraf* have been classified and granted market approval as devices. As engineered tissue products become less "structural" and more "functional" in nature, a "*device*" classification may become more difficult to square with the current statutory definition, although a product sponsor's desire to obtain this classification for its product may be undiminished.

Depending upon the manner of marketing approval, a tissue product classified as a device may be insulated from product liability litigation, while no such protection by reason of FDA review is available for tissue products classified as biologics. More immediately, only products classified as drugs or biologics are subject to the Prescription Drug User Fee Act. Under this act, sponsors of biologics are assessed fees in excess of $250,000 in conjunction with the filing and review of an application for marketing approval; sponsors of devices do not pay such fees. On the other hand, certain biologics may qualify for a special product designation that may waive the user fee payment and provide other benefits not otherwise available for devices.

In most cases, the classification of an engineered tissue product is effectively predetermined by the nature of the product itself and the manner in which it is intended to convey a therapeutic benefit. Nevertheless, consideration should be given to the greater implications of product classification early in the development process and certainly before discussing applicable methods of regulatory review with the FDA.

Special Product Designations

The FD&C Act recognizes that demand for all new medical products is not equally large or robust, such that the cost of obtaining marketing approval for a given product may be prohibitive in view of the relatively small size of the population it will benefit. To reduce the likelihood that a financial cost-benefit analysis applied to rarer diseases will leave them untreated, the FDA is authorized to grant special considerations and exceptions to reduce the economic burden upon developers of products under such conditions. Thus, the FDA may be petitioned to grant a "humanitarian device exemption" for certain devices (FD&C Act, §520(m)) or to recognize certain drugs or biologics as "orphan drugs" (FD&C Act, §525, et. seq.). However, the significance or value of these designations—especially for sponsors of tissue products—varies considerably according to the classification of the product in question.

Humanitarian use devices are those intended to treat a disease or condition that affects fewer than 4,000 people in the United States. The FDA is authorized to exempt a sponsor from the obligation to demonstrate the effectiveness of such a device to obtain marketing approval; however, the sponsor is precluded from selling the product for more than the cost to develop and produce it.

Orphan drugs are those intended to treat a disease or condition affecting fewer than 200,000 persons in the United States, or for which there is little likelihood that the cost of developing and distributing it in the United States will be recovered from sales of the drug in the United States. The orphan drug designation was established through an amendment of the FD&C Act by the 1982 Orphan Drug Act (ODA) prior to the creation of the humanitarian device exemption. In contrast to the humanitarian use devise designation, the orphan drug designation could be important to sponsors of certain engineered tissue products classifiable as biologics, illustrating the larger implications of the classification process. An orphan drug is defined to include biologics specifically licensed under §351 of the PHS Act, a distinction which may be relevant under the FDA's proposed plan for regulating engineered tissue products (see below). The FDA is empowered, under certain conditions, to grant marketing exclusivity for an orphan drug in the United States for a period of seven years from the date the drug is approved for clinical use; this exclusivity is stronger than and far less expensive to maintain than that provided by a patent. Additional benefits of the orphan drug designation include: certain tax credits for clinical research expenses; cash grant support for clinical trials; and waiver of the expensive prescription drug filing fee. A petition for orphan drug designation must be filed before any application for marketing approval.

Human Cellular and Tissue-Based Products

Human tissues used for medical purposes that have been regulated by the FDA as products have been classified as devices (including dura mater, human lenticules, and allograft heart valves) or as biologics (including blood, blood components, and blood products) (see Figure 8.1). Consequently, engineered human "tissue products" can be expected to be regulated by the FDA under these classifications as well (with at least the possibility of classification as a drug), although the criteria and process for such classification and subsequent marketing review will be substantially influenced by new regulations that the FDA is developing for cellular and tissue-based products.

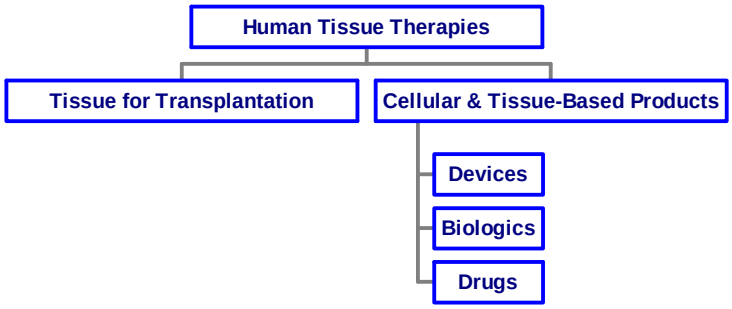


Fig. 8.1. FDA classification of therapeutic human tissue therapies.

In October 1993 the FDA announced that it considered its existing statutory authority mandated its regulation of autologous or allogeneic cells that have been propagated, expanded, selected, pharmacologically treated, or otherwise altered in their biological characteristics *ex vivo*, and intended to be administered to humans for the prevention, treatment, cure, diagnosis, or mitigation of disease or injuries (58 Federal Register 53248; October 14, 1993). The FDA also announced that the same statutory authority would extend to gene therapy products containing genetic material administered to modify or manipulate the expression of genetic material in order to alter the biological properties of living cells. The announcement explained that the FDA expected such somatic cell and gene therapy products would be classifiable as biologics subject to then-existing product and establishment licensure requirements (since consolidated under the current biologics license), but it noted that drug and device classifications could also be applicable.

A few months later, the FDA announced proposed rulemaking with regard to the acquisition and distribution of human tissue intended for transplantation (58 Federal Register 65514; Dec. 14, 1993). In contrast with its approach to somatic and gene therapies, the FDA did not claim transplanted tissues would be regulated as medical products. Instead, persons involved in the transfer of these tissues would be subject to donor screening, record-keeping, and processing standards pursuant to the FDA's authority under §361 of the PHS Act to prevent the spread of communicable diseases.

Much of the regulatory framework for engineered tissues is now being promulgated by the FDA through formal, binding, rule-making procedures. Previously, the FDA had issued a number of documents which, while not binding upon the Agency, did provide the public with a formal expression of its evolving thinking regarding the future regulation of human cellular or tissue-based products (see Table 8.1). Of these documents, by far the most important has been the *Proposed Approach to Regulation of Cellular and Tissue-Based Products* ("Proposed Approach") that the FDA issued on February 28, 1997.

Building upon the concepts and strategies set out in the Agency's 1993 pronouncements regarding somatic cell therapies and transplanted tissues, its Proposed Approach outlines a plan of regulatory oversight, which may include a pre-market approval requirement, for such tissue products based upon a matrix ranking the products, classified by certain characteristics, within identified areas of regulatory concern. These tissue products would be classified according to the relationship between the donor and the recipient of the biological material used to produce the tissue product; the degree of *ex vivo* manipulation of the cells comprising the tissue product; and whether the tissue product is intended for a homologous use, for metabolic or structural purposes, or is to be combined with a device, drug, or another biologic (see Figure 8.2).

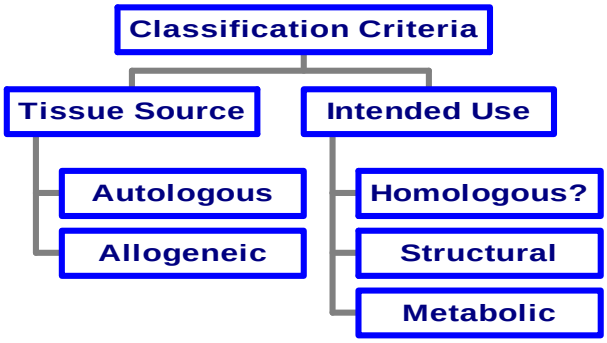


Fig. 8.2. FDA classification criteria.

The Proposed Approach also announced the establishment of an inter-Center Tissue Reference Group to act as an ombudsman to resolve product classification disputes and assure Agency-wide consistency in the application of relevant regulatory authority over harvested or engineered tissues used as medical therapies.

Since issuing the Proposed Approach almost five years ago, the FDA has been working to formalize its regulation of human tissue and cell therapies through a rulemaking process to amend the U.S. Code of Federal Regulations ("CFR") (see Table 8.1).

Table 8.1*
Key FDA Documents Concerning Regulation of Human Tissue and Cell Therapies

1. FDA Notice: *Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products* (58 FR 53248; Oct. 14, 1993).
2. FDA Notice of Interim Rule: *Human Tissue Intended for Transplantation* (58 FR 65514; Dec. 14, 1993).
3. FDA Notice of Public Hearing: *Products Comprised of Living Autologous Cells Manipulated ex vivo and Intended for Implantation for Structural Repair or Reconstruction* (60 FR 36808; July 18, 1995).
4. FDA Final Rule: *Elimination of Establishment License Application for Specified Biotechnology and Specified Synthetic Biological Products* (61 FR 24227; May 14, 1996).
5. FDA Notice: *Availability of Guidance on Applications for Products Comprised of Living Autologous Cells. . .*(etc.) (61 FR 26523; May 28, 1996).
6. *FDA Guidance on Applications for Products Comprised of Living Autologous Cells Manipulated ex vivo and Intended for Structural Repair or Reconstruction.*
7. FDA *Proposed Approach to Regulation of Cellular and Tissue-Based Products* (February 28, 1997).
8. FDA *Notification of proposed regulatory approach regarding cellular and tissue-based products* (62 FR 9721; March 4, 1997).
9. FDA Final Rule: *Human Tissue Intended for Transplantation* (62 FR 40429; July 29, 1997).
10. FDA Notice: *Availability of Guidance on Screening and Testing of Donors of Human Tissue Intended for Transplantation* (62 FR 40536; July 29, 1997).
11. FDA Guidance to Industry: *Screening and Testing of Donors of Human Tissue Intended for Transplantation* (July 29, 1997).
12. FDA Guidance for Industry: *Guidance for Human Somatic Cell Therapy and Gene Therapy* (March, 1998).
13. FDA Proposed Rule: *Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products* (63 FR 26744; May 14, 1998).
14. FDA Proposed Rule: *Suitability Determination for Donors of Human Cellular and Tissue-Based Products* (64 FR 52696; September 30, 1999).
15. FDA Proposed Rule: *Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products; Inspection and Enforcement* (66 FR 1508; January 8, 20041).
16. FDA Final Rule: *Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing* (66 FR 5447; January 19, 2001).

* With the exception of Document #1, each document listed here can be obtained through the FDA website (www.fda.gov/cber). While provisions of the FD&C and PHS Acts and the Final Rules, codified as part of the Code of Federal Regulations (CFR), promulgated thereunder by the FDA, have the force of law and are binding on the agency, FDA guidance documents are not. Nevertheless, Guidances are clearly helpful in anticipating the Agency's response to particular marketing approval and other regulatory issues.

Marketing Review and Approval Pathways

As discussed above, the particular program(s) of regulatory review applicable to a medical product are predetermined according to its FDA classification. Thus, the FD&C Act requires a sponsor to submit a device *Pre-Market Application* (PMA) or *Product Development Protocol* (PDP) to market a device, or a *new drug application* (NDA) to market a drug. The PHS Act provides that marketing approval for a biologic shall be obtained through the submission of a *Biologics License Application* (BLA). Certain drugs or biologics may qualify for special designation as orphan drugs under the Orphan Drug Act.

In addition, the FDA requires that sponsors of regulated products must first obtain preliminary approval for the clinical trials on humans that will support a subsequent application for full marketing approval. Clinical trials in support of a PMA application or as part of a PDP for a device may be conducted only after the FDA has issued an investigational device exemption (IDE); clinical trials in support of an application for

marketing approval of a drug or biologic cannot be initiated until the FDA has approved an investigational new drug (IND) application.

Devices

The FDA has divided devices into three classes to identify the level of regulatory control applicable to them. The highest category, Class III, includes those devices for which pre-market approval is or will be required to determine the safety and effectiveness of the device (21 CFR, §860.3(c); 21 U.S.C., §360c(a)(1)(C)). Absent a written statement of reasons to the contrary, the FDA classifies any "implant" or "life-supporting or life-sustaining device" as Class III (21 CFR, §860.93; 21 U.S.C., §360c(c)(2)(C)).

There are two primary pathways by which the FDA permits a medical device to be marketed: pre-market clearance by means of a 510(k) notification, or pre-market approval by means of a PMA or PDP submission.

A sponsor may seek clearance for a device by filing a 510(k) pre-market notification with the FDA, which demonstrates that the device is "substantially equivalent" to a device that has been legally marketed or was marketed before May 28, 1976. The sponsor may not place the device into commercial distribution in the United States until the FDA issues a substantial equivalence determination notice. This notice may be issued within 90 days of submission but usually takes longer. The FDA, however, may determine that the proposed device is not substantially equivalent, or require further information such as additional test data or clinical data, or require a sponsor to modify its product labeling, before it will make a finding of substantial equivalence.

If a sponsor cannot establish to the FDA's satisfaction that a new device is substantially equivalent to a legally marketed device, it will have to seek approval to market the device through the PMA or PDP process. This process involves preclinical studies and clinical trials to demonstrate that the device is safe and effective.

FDA regulations (21 CFR, §860.7(d)) provide that, based on "valid scientific evidence," a device shall be found to be "safe:"

> . . . when it can be determined . . . that the probable benefits to health from use of the device for its intended uses and conditions of use . . . outweigh any probable risks[,]

and that a device shall be found to be "effective:"

> . . . when it can be determined . . . that in a significant portion of the target population, the use of the device for its intended uses and conditions of use . . . will provide clinically significant results.

Testing in humans to obtain clinical data demonstrating these qualities in support of a PMA or pursuant to a PDP must be conducted pursuant to an investigational device exemption. The IDE is the functional equivalent of the IND that governs clinical trials of drugs and biologics. As with other medical products, clinical testing is typically conducted in multiple phases, with the earliest phases primarily intended to demonstrate safety and later phases addressing both safety and efficacy considerations. The sponsor of the device must also demonstrate compliance with applicable current good manufacturing practices (cGMPs, now also known as Quality System Regulations) before the FDA may approve the product for marketing by granting the PMA or accepting the completion of the PDP.

The Product Development Protocol. The 1976 Medical Device Amendments (MDA) to the FD&C Act included a section which provided the sponsor of a Class III device with two product approval pathways, the PMA or the PDP. The legislative history of the MDA reveals an expectation within Congress that most Class III devices would be approved by the FDA in response to a PMA. Nevertheless, in providing the PDP alternative, faster development of innovative devices could be achieved, and certain sponsors, especially small device sponsors, would benefit from an approval process that merged the investigation of the device and the development of the information necessary for its approval into one regulatory mechanism. The conventional device approval model—the linear process of clinical investigation followed by premarket approval application—provides for little to no interaction between the sponsor and the FDA once an IDE has

been granted. Anticipating that many medical devices are subject to frequent modification during development and that small device sponsors, in particular, may lack the financial resources to repeat or rework clinical trials to bolster perceived deficiencies in a PMA, the drafters of the 1976 MDA added the PDP process.

The PDP process replaces the linear PMA model with an early, collaborative interaction between product sponsor and FDA to produce a focused clinical development plan that both parties anticipate will satisfy the statutory requirements for proof of safety and effectiveness within an established timeframe. In addition, the PDP process allows for modification of the development plan in consultation with FDA reviewers (or in accordance with established guidance) to assure that the development plan as revised, or the device or modified device, will obtain prompt approval upon submission of a notice of completion of the PDP at the conclusion of the clinical trial(s) contemplated under the PDP plan.

The PDP process is not an alternative to the PMA process in the sense that the statutory requirements for proof of safety and effectiveness are relaxed; rather, it incorporates the clinical development and regulatory review elements of the IDE-PMA process within a framework that can efficiently manage deviations from the original plan made necessary by experience. In addition, a PDP may demonstrate to prospective investors of an emerging biomedical company that a clear, predictable plan and timetable exists for achieving marketing approval for products upon which the company's future revenues and profitability may depend.

Biologics

Until recently, permission to market a biologic required two applications: one to obtain a product license application (PLA) for the biologic itself and another for approval of the facility where the biologic would be prepared, that is, an establishment license application. The 1997 FDA Modernization Act amended the PHS Act by eliminating the separate product and establishment license applications in favor of a single *biologics license application* (BLA), which, like the PMA or PDP for devices, includes an evaluation of compliance with appropriate quality controls and current cGMP as part of the assessment of the safety and efficacy of the product in question.

§351 of the PHS Act directs the FDA to approve a BLA on the basis of a determination that the biologic in question is "safe, pure, and potent." Those terms are defined in FDA regulations promulgated to give effect to that statutory authority:

> . . . *safety* means the relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time[;]
>
> . . . *purity* means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product . . . [and] includes but is not limited to relative freedom from residual moisture or other volatile substances and pyrogenic substances[;]
>
> . . . *potency* is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

Testing in humans to obtain clinical data demonstrating these qualities in support of a BLA must be conducted pursuant to an investigational new drug application. The IND is the functional equivalent of the IDE that governs clinical trials of devices. As with other medical products, clinical testing is typically conducted in multiple phases, with the earliest phases primarily intended to demonstrate safety, and later phases intended to address both safety and efficacy considerations.

The emphasis given to process by the earlier requirement of a separate approval of the manufacturing facility illuminates the dual nature of the regulatory authority created under the PHS Act and ultimately exercised by the FDA. Besides assuring that only safe, pure, and potent biologics are marketed in the United States, the FDA is also charged with a general duty to prevent the introduction, transmission, or spread of

communicable disease (PHS Act, §361(a)). While the BLA is an amalgam of product and process quality criteria, a particular emphasis upon the authority to eliminate sources of dangerous infection reappears in the context of the FDA's proposed regulatory triage for engineered tissues.

Human Cellular and Tissue-Based Products

In introducing the February 1997 "Proposed Approach," the FDA identified five areas of regulatory concern raised by the development of new medical products derived from the manipulation of human biological materials: communication of infectious disease; processing and handling; clinical safety and efficacy; indicated uses and promotional claims; and monitoring and education.

The FDA has proposed that autologous tissue that is banked, processed, or stored should be tested for disease, and it will require companies to keep appropriate records to assure that patient tissues are not mismatched or commingled. The Agency proposes that allogeneic tissue be tested for disease, that donors be screened, and that appropriate records be kept, although the extent of the required testing or screening will not be as great for nonviable tissue. Periodic submissions to the Agency showing compliance with the testing or record-keeping requirements will not be necessary; the FDA assumes that a company's observation of these requirements will be assured through the accreditation they can be expected to maintain with professional tissue banking or processing societies.

The extent of the FDA's proposed regulatory intervention in the areas of processing and handling and clinical safety and efficacy vary according to the characteristics of the particular tissue product in question. To the extent that a tissue product undergoes more than minimal manipulation in processing, is intended for a nonhomologous use, is combined with nontissue components, or is intended to achieve a metabolic outcome, the Agency will require a greater demonstration of safety and efficacy through appropriate clinical trials.

"Manipulation," in the Agency's Proposed Approach, is a measure of the extent to which the biological characteristics of a tissue have been changed *ex vivo*. The FDA has stated it presently considers cell selection or separation, or the cutting, grinding, or freezing of tissue, to constitute minimal manipulation. Cell expansion and encapsulation are examples of more than minimal manipulation.

To the extent that the tissue product only undergoes minimal manipulation, is intended for a homologous application to achieve a structural outcome (or reproductive or metabolic outcome, as between family members related by blood), and does not combine with non-tissue components, the FDA will expect "good tissue practices" to be observed but will not impose any reporting duties or, consistent with its authority under §361 of the PHS Act, any product licensing or pre-market approval requirements. Any other tissue product requires submission of appropriate chemistry, manufacturing, and controls information and BLA approval for any tissue product that does not incorporate nontissue components. Tissue products that are combinations of tissue and devices or tissue and drugs may be regulated according to established pre-market approval (PMA or PDP) or new drug application (NDA) schemes.

The FDA has announced its intention to initiate formal rule-making to establish binding regulations regarding cellular and tissue-based products. To that end, it has recently proposed regulations to compel the registration of sponsors and other persons engaged in production and distribution of such products.

OTHER CONSIDERATIONS RELEVANT TO ENGINEERED TISSUES

FDA Regulation and Product Liability

Protection from product liability lawsuits, in the form of an immunity from such litigation, may come from satisfying the federal regulations that govern the design and manufacture of, as well as the warnings to be provided with, medical products.

By virtue of the Supremacy Clause of the U.S. Constitution (Article VI, cl. 2), the federal government is permitted to regulate certain affairs free of state interference. State civil litigation is a form of regulation, so it is a form of interference. If Congress elects to exclusively regulate certain conduct, then litigation under

state law regarding the same conduct is prohibited, as it may produce inconsistent or conflicting standards regulating that conduct.

The public policy arguments in favor of federal preemption with respect to the regulation of medical products are readily discernible. While both state and federal regulation have the enhancement of public health and safety as their goals, establishment of nationwide labeling and design criteria for medical products promotes uniformity and regularity in the interpretation of applicable regulations and ensures that enforcement of these regulations is conducted in the public interest, rather than through isolated lawsuits that may produce inconsistent results. In addition, the natural preeminence of a federal administration administering such regulations simplifies and improves communication between the regulators and the medical product sponsors. Federal preemption, then, is not a shield for bad medical products; rather, it protects a process of reasoned, scientific inquiry.

Ownership of Human Tissues

Significant advances in medical research over the past several years have contributed substantially to the commercial utility of human biological materials. Consequently, the source of such materials used in the creation of engineered tissue products may become important for reasons beyond—and certainly removed from—the possible transfer of adventitious agents or the management of immunological responses. Simply put, the use of allogeneic materials raises issues of ownership, donation, and consent not to be found with respect to autologous tissues.

The common law of the United States recognizes a severely restricted property interest in human bodies or organs. In a broad sense, a “property interest” in something may be thought of as a “bundle of rights” to possess, to use, to profit from, to dispose of, and to deal in that thing. Courts have granted next of kin nothing more than a “quasi-property” right—or right of sepulcher—in a decedent's body for the purposes of burial or other lawful disposition. In place of an exegesis of the religious or cultural prohibitions against recognizing a property interest in a dead body, it is clear that the limited right that has been fashioned by the courts has been intended to offer nothing more than that some interested person may ensure the remains are disposed of with dignity.

The limited biological resources to support organ transplantation have certainly created the conditions for a market for human body parts. In response, Congress and state legislatures have enacted statutes prohibiting the sale of any human organ. The National Organ Transplant Act (42 U.S.C., §§273 *et seq*.) was passed to regulate the availability of organs for transplantation through voluntary donation exclusively by explicitly prohibiting organ purchases. The same prohibition has been passed into law by the 15 states, to date, that have adopted the Uniform Anatomical Gift Act (1987). Other state statutes have imposed criminal penalties for the purchase of organs or tissue from either living or cadaveric providers.

These federal and state statutes effectively banning purchases of human organs were enacted in the mid-1980s in immediate response to the prospect of a widespread trade in these body parts to supply the growing demand for transplant material. The vision of a vendor peddling livers and kidneys—or worse, a patient harvesting one of his own organs for money—clearly hovered over the debate leading to the passage of this legislation. But that vision imagined people self-dismantling for cash; it did not really allow for a trade in renewable body parts, especially cells.

Whether the law would also abhor the sale of naturally regenerating cells was answered in the affirmative by the 1990 decision of the California Supreme Court in *Moore v. Regents of University of California* (51 Cal.3d 120, 271 Cal.Rptr. 146, 793 P.2d 479, 1990). The plaintiff, John Moore, claimed he held a property interest in the T-lymphocytes that had been harvested by his physician when his spleen and other bodily substances had been removed in the course of treating his hairy-cell leukemia. The T-lymphocytes were subsequently used to develop a cell line capable of producing a potentially lucrative strain of lymphokines. The development of the cell line and the financial rewards to be reaped from it were not disclosed to Mr. Moore when he consented to the surgical procedures necessary to treat his disease. Mr. Moore sued his physician and others for, among other things, conversion of his tissues, including his spleen, blood and the cell line derived from his cells. The California Supreme Court rejected Mr. Moore's conversion action; it refused to concede to him a property interest in his excised cells.

In the years following the *Moore* decision, few courts in the United States have had occasion to give further consideration to the nature of donors' ownership interests in their tissues. However, in order to provide for the privacy of genetic information, legislation proposed in some state assemblies has suggested donors may have an economic interest in such information and, by inference, in the tissues from which it would be derived.

REGULATION OF PHARMACEUTICAL/MEDICAL HUMAN TISSUE PRODUCTS IN EUROPE

Regulation of medical products incorporating viable human tissue products among or within the member states of the European Union is marked by inconsistency but is presently the subject of substantial discussion and debate. As part of the overall coordination of national laws and governmental activities within the EU, the regulation of the marketing of certain medical products by national authorities is being consolidated within designated EU agencies, especially the European Medicines Evaluation Agency (EMEA).

Within the scope of what medical products are considered pharmaceutical and regulated, there are two broad subcategories, medicinal products and medical devices, as shown in Figure 8.3.

Table 8.2
Classification of Human Tissue Products by EU Member States

Product Type	Regulatory Classification		
	Pharmaceutical	**Unregulated**	**Other**
Viable allogeneic skin replacement	Austria; Denmark; Germany; Spain; Sweden; UK	Finland; Ireland; Italy; Netherlands	Belgium; France; Greece
Nonviable allogeneic skin replacement	Austria; Germany	Denmark; Finland; Ireland; Italy; Netherlands; Spain; UK	Belgium; France
Autologous implant	Austria; Germany; Sweden	Denmark; Finland; Ireland; Italy; Netherlands; UK	Belgium; France; Greece; Spain

Source: Allison Dale, Smith & Nephew

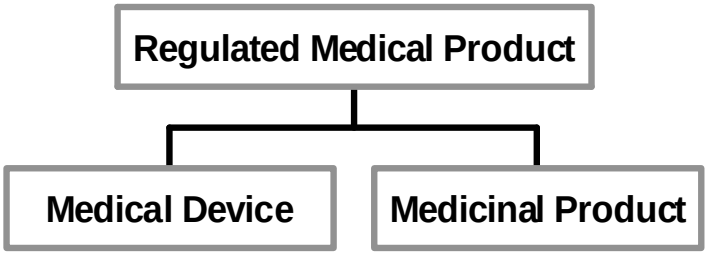


Fig. 8.3. European classifications of regulated medical products.

The EMEA was established in 1993 by the European Economic Community (EEC, now EU) Council Regulation No. 2309/93 to implement procedures to give effect to a single market for "medicinal products" among the member states. In conjunction with three directives adopted concurrently (Council Directives 93/39EEC, 93/40EEC and 93/41EEC), the regulation authorized EMEA to manage a "centralized procedure" for an EEC authorization to market medicinal products for either human or veterinary use. The directives also established a "mutual recognition procedure" for marketing authorization of medicinal products based upon the principle of mutual recognition of authorizations granted by national regulatory bodies. These procedures came into effect on January 1, 1995, with a three-year transition period until December 31, 1997. As of January 1, 1998, the independent authorization procedures of the member states are strictly limited to the initial phase of mutual recognition (i.e., granting marketing authorization by the "reference Member State") and to medicinal products that are not marketed in more than one member state. Consequently,

sponsors seeking marketing authorization for medicinal products throughout the EU are obliged to seek such approval through the centralized procedure administered by EMEA.

The concept of a "medicinal product" in EEC legislation substantially predated the organization of EMEA. Council Directive 65/65EEC of January 26, 1965, defined the term *medicinal product* to include

> any substance or combination of substances presented for treating or preventing disease in human beings or animals.
>
> [and]
>
> any substance or combination of substances which may be administered to human beings or animals with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in human beings or in animals ….

A "substance" is further defined to include "[a]ny matter irrespective of origin which may be human … animal … vegetable … [or] chemical" (Directive 65/65/EEC, Article 1). However, the directive also makes clear that its regulation of medicinal products (and, through amendments to the directive recognizing the authority of EMEA, the "centralized procedure") does not apply to products "intended for research and development trials" (Directive 65/65/EEC, Article 2).

Sponsors of medical products derived through tissue engineering have reported substantial inconsistency among the regulatory bodies of EU member states regarding the classification of such products for purposes of determining the applicability of national or EU marketing authorization requirements (see Table 8.2). A determination that engineered tissue products are "medicinal products" subject to the centralized procedure for authorization administered by EMEA will substantially clarify and rationalize the process by which such products may be marketed throughout the European Community.

The EMEA has in place a Biotechnology Working Party that has considered, among other things, safety issues in the delivery of human somatic cell therapies and a definition of a "cell therapy medicinal product" (CPMP/BWP/41450/98 draft). This definition would consider engineered human tissues to be "medicinal products" within the meaning of Directive 65/65/EEC, provided the engineered tissue was the product of both the following:

> a. …. an industrial manufacturing process carried out in dedicated facilities. The process encompasses expansion or more than minimal manipulation designed to alter the biological or physiological characteristics of the resulting cells, and
>
> b. further to such manipulation, the resulting cell product is definable in terms of qualitative and quantitative composition including biological activity.
>
> *(Points to Consider on Human Somatic Cell Therapy, CPMP/BWP/41450/98,* draft, page 3/9.)

The Biotechnology Sector of EMEA is likely to have primary responsibility for considering the authorization of engineered tissue products in the event they are classifiable as "medicinal products."

Human tissue and cellular products may not be presently definable as "medicinal products" subject to regulation, to the extent they are the result of modest manipulation of autologous tissues in the course of treating a fairly small patient population. Under these circumstances, the regulation of such cellular products is more likely to remain with the competent authorities of the Member States (with substantial variability in the classification and resulting regulation of such products, as outlined in Table 8.2). Nevertheless, an EMEA decision to accept an engineered tissue product as a "medicinal product" could occur in response to a petition from a sponsor of such a product. To be successful, such a petition should probably stress the "industrial" nature of the fabrication process and the extent of manipulation of the human biological material to produce the engineered tissue product. Assuming an engineered tissue product could be established to be a "medicinal product," there does not appear to be any EU rule that could limit the ability of EMEA to grant market authorization according to the type or source of tissue from which the product had been derived.

EMEA is aligned with Enterprise DG (formerly DG III; the department of the European Commission primarily responsible for establishing and implementing rules promoting the Single Market for products). A unit of Enterprise DG oversees application of EU directives regulating marketing authorization of medical devices. Providing for engineered tissue products could require some reconsideration of the specific areas of responsibility of the units or agencies involved in regulating medical products.

REGULATION OF PHARMACEUTICAL/MEDICAL HUMAN TISSUE PRODUCTS IN JAPAN

It appeared at the time of the WTEC panel's visit to Japan that the Government of Japan was only beginning to focus on codifying regulation of engineered human tissue products within its scheme of regulating other medical products. The WTEC panel was unable within the scope of this study to provide an analysis of Japan's medical product approval process as potentially applied to engineered human tissue products. However, presented here is an outline of Japan's process and agencies responsible for regulation of medical products generally.

The Pharmaceutical and Medical Safety Bureau (PMSB) has primary responsibility within the Japanese Ministry of Health, Labour and Welfare for administering the requirements established for the safety and efficacy of medical products under Japan's Pharmaceutical Affairs Law. This legislation was substantially amended in 1996 (with the reforms made effective in April 1997) to provide for the present medical product review and approval system.

Applications for approval of new drugs and medical devices are referred by PMSB to the Central Pharmaceutical Affairs Council (CPAC) to obtain its recommendation. The CPAC, in turn, is advised by the Pharmaceutical and Medical Devices Evaluation Center (PMDEC), an expert body organized in July 1997 to evaluate the quality, efficacy, and safety of medical products administered to humans. Specific authority within PMSB to approve recommendations received from CPAC regarding the discrete aspects of the clinical testing, licensing, and use of new medical products is distributed among relevant divisions, such as the Evaluation and Licensing Division (pre-marketing and supplemental application approvals) and the Safety Division (adverse reaction measures). A regulatable medical product in Japan is classified as either a medical device or a pharmaceutical (Figure 8.4).

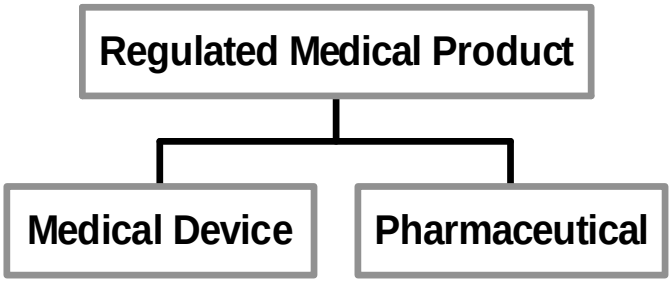


Fig. 8.4. Japanese classification of regulated medical products.

Advice concerning the design and conduct of clinical trials, as well as the adequacy of applications for approval of pharmaceuticals, is provided to PMDEC and to the product sponsor by the Drug Organization, a quasi-governmental agency established in 1979 as a fund to support patients experiencing adverse drug reactions. It is not clear whether the Drug Organization serves a similar function with respect to medical devices, or if there exists an equivalent medical device organization. However, applications for approval of "copy-cat" devices are referred to the Japan Association for the Advancement of Medical Equipment for a determination of the equivalence of the new device to devices already approved for clinical use. For a more detailed description of Japan's general medical product approval process, see, for example, Hirayama 1998 and Yamada 1997.

CONCLUSION

No part of the process of bringing new biomedical products from the laboratory to the patient occurs in isolation from or independent of all of the other aspects of organizing and maintaining that technology development effort, including intellectual property protection and financing, just to mention two. While pre-market approval is the most obvious form of external control over the introduction of new medical products in any country, it is not the only one. Healthcare reimbursement regulations and private insurer practices are critical components of establishing market acceptance. The approach to regulatory oversight itself requires careful analysis of product classification (including special designation) options. The novelty, variety, and potential complexity of forms of tissue engineering compel strategic analysis of external controls over the commercial development of human cellular and tissue-based products.

Regulatory issues present a major challenge to the worldwide development of the tissue engineering industry. The FDA approach to the regulation of products incorporating human tissues is comprehensive but not fully implemented. In the absence of an EU regulatory program, those European governments that have addressed the status of engineered tissue products have employed an array of classification schemes that further complicate international application of tissue engineering technologies. Like a number of European states, Japan has yet to articulate its own regulatory policies.

The implications of governmental authority over access to human tissues for research purposes are equally clouded by multiple responses to the legal, ethical, and cultural issues presented, with the recent debate over the use of embryonic stem cells highlighting these different approaches. Tissue engineering can proceed along two paths: the management of the natural process of proliferation and differentiation from the embryonic stage to produce only the specific tissues required; or the manipulation of differentiated somatic cells or partially differentiated stem cells to build functioning tissues. With the introduction of the additional ethical, cultural, and legal issues that attend upon the nontherapeutic experimentation on embryonic tissues, what might otherwise be simply a scientific debate has become an intensely political one.

Taken as a whole, this WTEC study's examination of legal and regulatory issues revealed the following:

- In comparison with the rapid progress being made to establish the therapeutic potential of human cellular and tissue-based strategies, the legal transfer and subsequent status of human tissues for research and product development is not well articulated, even within the United States. The result is that commercial development of engineered tissue therapies may be determined as much by tissue access and regulatory approval pathway as by clinical outcome.
- The pace and direction of the development and clinical introduction of engineered tissue products can be affected by many federal agencies.
- A general disengagement of the biomedical community from the policy-making processes of these agencies can deprive them of an important perspective on proposed actions.
- As the U.S. FDA evolves its strategy for managing engineered tissue products, it should emphasize cross-Center consistency in product classification and product approval paradigms that respond to the particular attributes and challenges of products incorporating living human tissues. The FDA's effort to develop a rational approach to the regulation of engineered tissue products is well begun; it should be continued and expanded globally through international harmonization programs.

REFERENCES

Emea Biotechnology Working Party, *Points to Consider on Human Somatic Cell Therapy*, *CPMP/BWP/41450/98* draft.

Food, Drug, and Cosmetic Act (as amended by the FDA Modernization Act of 1997). See also http://www.fda.gov/opacom/laws/fdcact/fdctoc.htm.

U.S. Food and Drug Administration Notice: *Application of current statutory authorities to human somatic cell therapy products and gene therapy products* (58 Federal Register 53248; Oct. 14, 1993).

U.S. Food and Drug Administration Notice of Interim Rule: *Human tissue intended for transplantation* (58 Federal Register 65514; Dec. 14, 1993).

U.S. Food and Drug Administration Notice of Public Hearing: *Products comprised of living autologous cells manipulated ex vivo and intended for implantation for structural repair or reconstruction* (60 Federal Register 36808; July 18, 1995).

U.S. Government. *Food and Drug Administration Modernization Act of 1997*. Public Law 105-115. See also http://www.fda.gov/cder/guidance/105-115.htm.

Hirayama, Y. Changing the review process: The view of the Japanese Ministry of Health and Welfare. 32 *Drug Information Journal* 111-117 (1998).

PHS Act. Effective 1999. Public Health Service Act, 42 U.S. Code Section 201. See also FDA website: http://www.fda.gov/opacom/laws/phsvcact/phsvcact.htm.

Uniform Anatomical Gift Act of 1987, Section 10. reproduced at 8A Uniform Laws Annotated 58 (Master Edition, 1993).

Yamada, M. The approval system for biological products in Japan. 31 *Drug Information Journal* 1385-1393 (1997).

APPENDICES

APPENDIX A. BIOGRAPHIES OF PANELISTS

Larry V. McIntire (Panel Chair)
E.D. Butcher Professor and Chair
Institute of Biosciences and Bioengineering
Rice University
MS 144, 6100 Main Street
Houston, TX 77005-1892

Larry V. McIntire is the E.D. Butcher Professor of Chemical and Biomedical Engineering at Rice University. He is also chair of the Institute of Biosciences and Bioengineering and chair of the Department of Bioengineering. Dr. McIntire received his BChE and MS degrees from Cornell University in 1966 and his PhD from Princeton University in 1970, all in chemical engineering. He has been at Rice University since 1970. His research interests include the effects of flow on mammalian-cell metabolism, molecular mechanisms of cell adhesion, tissue and cellular engineering, and bioengineering aspects of vascular biology. Dr. McIntire was the recipient of a National Institutes of Health MERIT Award for 1989-1999 and is a founding fellow and past president of the American Institute of Medical and Biological Engineering. He is past president and senior member of the Biomedical Engineering Society and past president of the North American Society of Biorheology. Dr. McIntire was the 1992 recipient of the American Institute of Chemical Engineering Food, Pharmaceutical, and Bioengineering Division Award; chair of that division in 1998; elected a fellow of that institute in 1994; was the 1992 ALZA Distinguished Lecturer for the Biomedical Engineering Society; and was a Sigma Xi National lecturer for 1993-95. In 1998, he was elected a fellow of the American Association for the Advancement of Science. Dr. McIntire was elected to the National Academy of Engineering in 2001.

Howard P. Greisler
Department of Surgery
Department of Cell Biology, Neurobiology, and Anatomy
Loyola University Medical Center
2160 South First Avenue
Maywood, IL 60153

and

Hines VA Hospital
Office of Research and Development
5th Ave. and Roosevelt Road
Hines, IL 60141

Dr. Greisler is both a practicing vascular surgeon and an active investigator in vascular biology and growth factory delivery for vascular tissue engineering. He is a graduate of the University of Pennsylvania and the Pennsylvania State University School of Medicine. He received training in surgery with fellowships in Vascular Surgery and Transplantation at the Columbia Presbyterian Medical Center in New York City. He is currently both professor of surgery and professor of cell biology, neurobiology, and anatomy at Loyola University Medical Center in Maywood, Illinois. Dr. Greisler has received numerous grants from the National Institutes of Health and the Veterans Administration and has served as a member of study sections for grant review at the NIH, the American Heart Association, and the Veterans Administration, and he has served on five editorial boards. He is the current president of the International Society for Applied Cardiovascular Biology, an honorary fellow of the Royal College of Surgeons of Edinburgh, chairman of the Lifeline Foundation Research and Education Committee, and he holds three U.S. and international patents. He has authored over 255 publications, including 4 books, and has given over 280 scientific and clinical presentations.

Dr. Greisler divides his time equally between research and clinical activities. His research endeavors are in the areas of vascular tissue engineering and angiogenesis, with specific attention to the regulation of growth factor secretion by arterial wall cells, local delivery of cytokines and growth factor to the vessel wall and to vascular grafts, and the role of these growth factors in modulating endothelial cell and smooth muscle cell proliferation.

Peter C. Johnson
President and Chief Executive Officer
TissueInformatics, Inc.
711 Bingham St., Suite 202
Pittsburgh, PA 15203

A native of Buffalo, NY, Dr. Johnson attended the University of Notre Dame (BS, 1976), received his MD degree from the State University of New York Health Sciences Center at Syracuse (1980), and completed general surgery training at Case-Western Reserve University (1987) and plastic surgery training at the University of Pittsburgh (1987). He also completed a research fellowship at Harvard University (1982-1985) in the study of thrombosis, cell biology, and biomaterials under the direction of Dr. Edwin Salzman. As a faculty member in the Division of Plastic Surgery at the University of Pittsburgh (1989-1998), Dr. Johnson served as research director, began and administered the multidisciplinary Facial Nerve Center, and was a practicing reconstructive microsurgeon. He presently serves as an adjunct associate professor of surgery at the University of Pittsburgh.

In 1994, Dr. Johnson began the Pittsburgh Tissue Engineering Initiative (PTEI), a unique collaboration between five regional research institutions and seven foundations that became a nonprofit corporation in 1996. The PTEI (http://www.pittsburgh-tissue.net) supports the development of novel patient care technologies in the emerging field of tissue engineering while providing educational and outreach services to scientists and the public. Dr. Johnson left clinical practice in 1997 to serve as full-time president of the PTEI. In January 1999 he became the chief executive officer of one of the Pittsburgh region's first biotechnology companies, TissueInformatics, which is designed to support the development of tissue engineering and other tissue-based companies worldwide through the provision of tissue structural information.

Dr. Johnson is the immediate past chairperson of the Plastic Surgery Research Council, the president of the Pennsylvania Biotechnology Association, the president of the Tissue Engineering Society International, and the chairperson of the American Society for Testing and Materials Committee F4, Division IV on Tissue Engineered Medical Product Standards. He also provides service as a member of the executive committee of the Carnegie Science Center. Dr. Johnson lives in Wexford, PA, with his wife, Karen, and three children, Caroline, Thomas, and Elizabeth.

David J. Mooney
Department of Chemical Engineering,
Department of Biologic and Materials Sciences
University of Michigan
2300 Hayward St.
Room 3074 H.H. Dow Bldg.
Ann Arbor, MI 48109-2136

Dave Mooney received his chemical engineering education from the University of Wisconsin (BS 1987) and MIT (PhD 1992). He subsequently was a post-doctoral fellow at Harvard Medical School. He joined the faculty of the University of Michigan in 1994. He is presently an associate professor of chemical engineering, biomedical engineering, and biologic and materials sciences. His research interests are in biomaterials and tissue engineering. His laboratory studies the mechanisms by which cells receive signals from the materials they contact and utilize this information to design and synthesize polymers to deliver inductive proteins, plasmid DNA, and cells in order to engineer new tissues. He has published over 100 research articles, book chapters, and review articles in the tissue engineering and mechanotransduction areas. He has won several awards, including the NSF Career Award and the NIH FIRST Award. He is a founding member of the Tissue Engineering Society, and has served as scientific officer and chair of the program committee for this society. Dr. Mooney is also a director of the Materials Science and Engineering Division of AIChE and has organized symposium and topical conferences at MRS, ACS, AIChE, and Society for Biomaterials national meetings. He currently serves on the editorial boards of *Tissue Engineering*, *Journal of Dental Research*, *Regenerative Medicine*, and *Biotechnology Progress*.

Milan Mrksich
Dept of Chemistry
SCL-463
University of Chicago
5801 S. Ellis Ave.
Chicago, IL 60637

Milan Mrksich is associate professor of chemistry at the University of Chicago. He received degrees in chemistry from the University of Illinois (BS, 1989) and the California Institute of Technology (PhD, 1994). He was a postdoctoral fellow at Harvard University for two years and then joined the faculty at the University of Chicago. His research interests are in surface chemistry and tailored bio/materials interfaces. His research group is using model substrates that present peptide and carbohydrate ligands for mechanistic studies of cell adhesion and migration. His group has also developed routes towards dynamic substrates that can alter the presentation of ligands under electrochemical control and is applying these active substrates to chip-based systems. Dr. Mrksich serves as a frequent consultant to biotechnology companies and currently serves on the scientific advisory boards of Cellomics, ChemoCentryx, and Surface Logix, and on the Defense Sciences Research Council.

Nancy L. Parenteau
Senior Vice President and Chief Scientific Officer
Organogenesis, Inc.
150 Dan Road
Canton, MA 02021

Dr. Parenteau is senior vice president and chief scientific officer at Organogenesis in Canton, Massachusetts. Organogenesis was one of the first companies dedicated to the field now known as tissue engineering. A New Hampshire native, Dr. Parenteau received her BA in zoology from the University of Vermont and a PhD in anatomy from Georgetown University, where she focused on cell and developmental biology. Her research involved the use of lymphocyte hybridoma technology to elucidate the ontogeny of molecular markers in the developing pancreas. This was followed by a postdoctoral fellowship at the Harvard School of Public Health, where she studied human keratinocyte regulation and differentiation. She joined Organogenesis in November of 1986 as group leader in Cell Biology. Dr. Parenteau later served as co-director of research and director of the Living Skin Equivalent Program and Cell Biology before becoming vice president of Cell and Tissue Science in 1994. During these years, she helped develop a living skin construct for *in vitro* and clinical use. This bi-layered organotypic construct of human skin is achieved using two types of living human cells, purified collagen, and specialized cell culture technology. She has been chief scientific officer since 1995.

Dr. Parenteau presently serves on the boards of the Pittsburgh Tissue Engineering Initiative and the Harvard-Forsyth Cranio-Facial Research Center. She is also a member of the Chemical Sciences Roundtable of the National Research Council.

David Smith
General Counsel
TissueInformatics, Inc.
711 Bingham St., Suite 202
Pittsburgh, PA 15203

David Smith is General Counsel of TissueInformatics, Inc. Previously, he was a counsel at Reed Smith Shaw and McClay, LLP, where he was primarily engaged in representing emerging biomedical companies, especially in the field of tissue engineering. He has written and lectured extensively on understanding and managing external controls influencing the development of new biomedical products and on the acquisition and use of human tissues and biological information.

Mr. Smith is a board member of the Pennsylvania Biotechnology Association; a board member and treasurer of the Tissue Engineering Society; chairman of the terminology subcommittee of Section F04. Div. IV

(Tissue Engineered Medical Products) of the American Society for Testing and Materials; and a member of the advisory council of the McGowan Center for Artificial Organ Development.

In 1977, Mr. Smith received his AB with honors from Hamilton College in Clinton, New York; and in 1980 he obtained his JD from American University Law School in Washington, D.C.

Mr. Smith has lived and traveled extensively overseas, especially in Great Britain and Japan. He and his wife, Carole Patton Smith, and their two daughters currently reside in Pittsburgh.

APPENDIX B. SITE REPORTS—EUROPE

Site: **bwA, Aachen Centre of Competence: Biomaterials**
Veltmanplatz 8
52062 Aachen, Germany
http://www.rwth-aachen.de/bwa

Date: 21 July 2000

WTEC Attendees: N.L. Parenteau (report author), S. Gould

Host: Prof. Dr. Hartwig Hoecker, Director, German Wool Research Institute
Tel.: +49-(0)241-4469-0; Fax: 49(0)241-4469-100
Email: hoecker@dwi.rwth-aachen.de

BACKGROUND

A center of competence established at the University of Technology (RWTH), Aachen, Germany, is bringing together basic research, materials, R&D, and industrial production in the field of biomaterials. The institution on which the center of competence was organized, the German Wool Research Institute, itself is over 50 years old and is engaged in research on wool, polymers, and proteins. It has 130 employees, including up to 80 PhD students.

The Center of Competence in Biomaterials, founded 4 years prior to this WTEC visit, has support from the Federal Ministry of Research and Education, scheduled to expire at the end of 2001. After that the center plans to continue with funding from industrial contracts. The center is composed of investigators in chemistry, biochemistry, pathology, and engineering (textiles, metals, ceramics). All researchers have close industrial relations and projects.

An interdisciplinary center for clinical research also was founded in Aachen, in 1992. It is focused on biomaterials and features cooperation between medical doctors and other faculty members at the university as well as, to some extent, with industry. Both the center of competence and the interdisciplinary center for clinical research are doing work related to tissue engineering.

The majority of the students involved in these programs (95%) enter industry post diploma. Many of Dr. Hocker's students have studied in the United States prior to or after their diploma. Dr. Hocker has sent several students to Dr. Langer's laboratory at MIT.

Approximately 15 years ago, a collaboration was started between biomaterials and pathology scientists to find biocompatible materials for an artificial blood vessel. This resulted in a polyurethane vessel with surface modifications to promote endothelialization. This reached clinical trials. It also focused the group on blood compatibility and began a clinical research relationship, which persists to this day. The center is responsible for advancing a number of materials to the clinic, often in collaboration with an industrial partner. It has many agreements with industry. An example is a textile biomaterial to promote better tissue integration of a keratoprothesis. The center has a particular expertise in textiles, having originally started as a wool research center. Another example of synergy of technologies was a poster on the development of a ligament using a braided biomaterial.

The interdisciplinary center for clinical research was established 8 years ago as a government (BMFT) initiative to motivate medical doctors to do medical research in the area of biomaterials. This led to the establishment of the Center of Competence in Biomaterials. This is one of four centers of competence funded in this way. These centers have limited funding, and it is anticipated that by 2002 they will have to become self-funded. Each center covers a non-overlapping area. Besides the biomaterials center, the other three are: (1) Rosteach (soft tissue), (2) Ulm (hard tissue), and (3) Denkendorf-Tubingen (soft tissue).

Because of Aachen's expertise in blood compatibility with surfaces, its scientists have a particular interest in the modification of traditional surfaces or polymers by means of protein, charge, peptide sequences, anti-thrombotic agents, and so on. Their primary approach is to use traditional polymers and segmented copolymers composed of not more than 5 different known monomers with established properties, combined in novel ways to achieve desired physical and biological properties and rates of degradation. They prefer scaffolds that degrade by erosion rather than something like PGA. They are then interested in combining bioactive molecules with these novel segmented copolymers; a wound scaffold was given as an example. They are also forming depsipeptides, which are alternating copolymers of lactic acid and amino acid. It is felt that these will significantly reduce the negative effects of polymer degradation, decreasing the acid formed. The blood compatibility group provides the cell biology and cell culture expertise for testing the bioactive polymers. There is a current collaboration with the University of Munster in connective tissue on a hernia repair patch.

INDUSTRY OVERVIEW

Novel Polymers

The approach of the Aachen researchers to novel polymers is to use traditional monomers combined in novel ways. They are also pursuing the depsipeptide approach to enhance the performance of degradable polymers. This is in contrast to a group such as J.A. Hubbel's, which is also exploring the modification of biological polymers. (Author's note: I cannot comment further on this, not knowing other work in this specific area.)

Surface Modification of Polymers

Having a blood compatibility background and expertise, this group has in earlier years advanced to the clinic using surface-modified polymers in the vascular graft work. Although no specifics were given, the impression is that this group has at least the basic chemical and biological expertise to be competitive in this area.

Funding Strategy and Technology-to-Market Issues

Funding for the center was to end in less than two years. The plan was to create a company at the center with a focus in three areas:

1. Continued support of research
2. Development of mechanisms for the formation of start-up companies to spin off center discoveries, with the center retaining an equity stake
3. Looking for patents that may be licensed or serve as a basis for start-up companies

PARADIGM SHIFTS

The biggest paradigm shift will be for the center to become less of a contract research house and more entrepreneurial in its approach. Previously, it did not retain an equity stake or royalties from any work done in collaboration with industry. It is not clear how the plan will be implemented, but one must be formulated to ensure future funding of the center.

REFERENCES

RWTH. 1999. *Annual report. Aachener Deutsches wollforschungsinstitut*

_____1998. *Textile conference*. DWI Reports 1-596.

Site: **Cell Lining GmbH**
Rudower Chausee 29 (OWZ)
12489 Berlin
Germany
http://www.cell-lining.de/

Date: 18 July 2000

WTEC Attendees: J. West (report author), S. Gould, F.G. Heineken, B. Wagner

Host: Dr. med. Manrico Paulitschke

BACKGROUND

The Cell Lining Company, founded in 1996, is located in the WISTA Technical Park in southeast Berlin. It currently sells primary human and animal cells (osteoblasts, chondrocytes, periosteal cells, keratinocytes, fibroblasts, smooth muscle cells, epithelial cells, and endothelial cells); culture medium; and cell perfusion systems (flow chamber, perfusion chamber, and vascular graft perfusion systems). It currently has R&D projects in the seeding of endothelial cells onto vascular grafts and heart valves as well as a tissue-engineered cartilage project. These are done in collaboration with Charité, primarily at the Charité site. Initial funding of Cell Lining was provided by government grants, and current funding is through venture capital. There are currently eight employees (2 working at Charité). The current facility is not GMP-certified, so the company was planning to move shortly. The vascular graft lining project (expected to be the first clinical product from Cell Lining) was being done in collaboration with Charité and the German Heart Institute.

The vision for this product is that a surgeon will send a forearm vein sample from the patient to a regional center where endothelial cells will be isolated and expanded. The appropriately sized vascular graft will be placed in a perfusion chamber and coated with a commercially available homologous fibrin glue product. Endothelial cells will then be seeded onto the coated graft. Shear stress will be gradually introduced, and finally pulsatile pumping under physiological conditions will be applied to "condition" the endothelial cell lining. Results suggest that after conditioning, the endothelial lining can be exposed to static conditions for up to 24 hours for shipment to the hospital.

R&D ACTIVITIES

Biomaterials

The company was conducting no research in this area and was using only commercially available materials.

Cells

Cell isolation and monolayer and three-dimensional culture is the main expertise of this company. Efforts are focused on applications utilizing autologous cells.

Engineering Design

Though the development of perfusion/bioreactor systems is a major focus of this company, none of the company's engineers are involved. The development of new systems is based on collaboration with an outside engineering company.

GOVERNMENT FUNDING

The government provided DM 1 million funding for initial start up.

REFERENCES

Cell Lining GmbH, Cell lining. Brochure.

_____. Cell and Tissue Culture Systems from Cell Lining. Flier.

_____. Perfusion Chamber PCS^{3c}. Flier.

_____. Tube Chamber TCS^{2c}. Flier.

Site:	**Berlin Workshop** **German Heart Institute** **Deutsches Herzzentrum Berlin (DHZB)** **Virchow Campus**
Date:	17-18 July 2000
WTEC Attendees:	F.G. Heineken (report author), S. Gould, J. West, W. Wagner
Host:	Dr. Günter Peine BioTOP http://www.biotop.de/ Dr. Gudrun Tiedemann, CEO BioTOP Dr. Michael Sittinger Humboldt University Dr. Martina Seifert Humboldt University Dr. Andreas Kage Humboldt University Biotechnologiepark Luckenwalde: http://www.bio-luck.de MeGA Tec GmbH: http://www.mega-tec.de

BACKGROUND

On the first day of the WTEC team's visit to Berlin, Dr. Günter Peine of BioTOP (http://www.biotop.de) arranged a workshop on tissue engineering for the site-visit team. This workshop took place at the Deutsches Herzzentrum Berlin (DHZB) (German Heart Institute) on the Virchow Campus in Berlin. After the workshop, Dr. Peine arranged a laboratory tour of the liver support facilities on the Virchow Campus. The topics in the workshop and laboratory visit covered all the major tissue engineering activities taking place in Berlin, including bone/cartilage, skin, vascular including coronary artery, heart valve, liver support, and hematopoiesis..

Dr. Gudrun Tiedemann, CEO of BioTOP, started the workshop with an overview of biotechnology in Germany. Since 1995, when the laws regulating biotechnology in Germany were liberalized, there has been tremendous growth in this field. Berlin and the surrounding state of Brandenburg now have the most biotechnology activity (commercial and academic) in Germany. With Brandenburg and East Berlin being part of the former East Germany, there is substantial federal government support for commercial development in this part of Germany; tissue engineering is an important component of the biotechnology activity there.

On the second day of our visit to Berlin, Dr. Peine arranged for us to visit an incubator facility called "Adlershof," which is located in the southeastern part of Berlin. This is where the laboratories of the former Academy of Sciences of East Germany were located, and where the current local and federal governments are providing for start-up companies and universities to share facilities. We visited one such company named "Cell Lining" (http://www.cell-lining.de), which specializes in coating polytetrafluoro ethylene (PTFE) vascular grafts with endothelial cells to make them more biocompatible and thromboresistant. Our host Dr. Paulitschke kindly discussed his research activities with us and showed us his laboratory facilities. Dr. Paulitschke was a participant in the previous day's workshop. Future plans call for university faculty from the Charité Medical School (of Humboldt University) to move into facilities to be constructed at Adlershof.

On the afternoon of our second day in Berlin, Dr. Peine took us to visit the Charité Medical School, which was very well known for its medical clinics and research before World War II. It is located in what was East

Berlin and very close to the Brandenburg Gate. We visited Dr. Sittinger there, who showed us his laboratory facilities and discussed his research activities with us. Dr. Sittinger also was a participant in the previous day's workshop.

HUMBOLDT UNIVERSITY (CHARITÉ)

Presentations were made to the WTEC group on bone/cartilage tissue engineering by Dr. Michael Sittinger, and on skin tissue engineering by Drs. Martina Seifert and Andreas Kage. All three investigators are on the faculty of the Charité Medical School of the Humboldt University (Charité). Dr. Sittinger is a member of the German Rheumatism Research Center at Charité. He is also the chief scientific contact for a new start-up company named "Trans Tissue Technology," which is to be located at the Charité facility, and which is to market bone/cartilage tissue-engineered products. Dr. Kage is also head of the scientific advisory board of a start-up company (MeGA Tec GmbH) in the area of skin tissue engineering. The company is located in an industrial park named the Luckenwalde Biotechnology Park, which is close to Potsdam. Founders of the company were Andreas Salomon and Doris Weitzel-Kage. Dr. Kage commented later that the company is installing a GMP-certified process for autologous cell transplantation, which was to be finished in December 2000.

Bone and Cartilage Tissue Engineering

Dr. Sittinger's research is focused on bone and cartilage tissue engineering. For purposes of placing his research activities in the context of the eight chapters of this WTEC report, his research can be summarized as follows.

Biomaterials

Some of the biomaterials used in Dr. Sittinger's research include hydrogels, hyaluron, autologous fibrin glues, and sponge materials.

Cells

Dr. Sittinger's main effort is in the culturing and co-culturing of osteoblasts and chondrocytes. Co-culturing is being done to promote adhesion of cartilage to bone, which is a major issue with load-bearing cartilage materials. Cells being used are primarily autologous.

Biomolecules

Some work is being done with bone morphogenic proteins to reduce inflammation caused by rheumatoid arthritis.

Engineering Design Aspects

Mechanical properties of the tissue-engineered bone and cartilage produced in Dr. Sittinger's laboratory are major parts of his research program. Non-load-bearing cartilage has been used for facial cosmetics in human patients. Studies on load-bearing cartilage are being carried out in rabbits and horses. As mentioned previously, attaching load-bearing cartilage to bone is a major problem. Dr. Sittinger is trying to address this problem by co-culturing chondrocytes and osteoblasts in a controlled system consisting of a sponge material (polyglycolic acid). Even when this is successful, transplantation is another major problem.

Informatics

All of the data being collected by Dr. Sittinger are digitized and stored in that form. These data are accessible via local area networking so that Dr. Sittinger has access to these data from his home computer via a telephone linkage. None of the results that were reported to us were based on mathematical modeling, but were empirically based.

Legal and Regulatory Issues

Carticel (Genzyme's cartilage product) has been approved for use in Germany. However, reimbursement from insurance plans is still a problem. It has not been decided whether Carticel is a device or a drug. There is no German-made cartilage product that has been approved for medical use. Germany needs to have a good manufacturing production (GMP-certified) facility before approval is a possibility.

Dr. Sittinger has filed for 10 patents based on his research activities. Some of these have already been issued and are the basis for the formation of his company.

Government Activities and Interests

Dr. Sittinger has been able to obtain funding for his research from the German government, which has also been very supportive of linking his research with commercialization of that research by allowing his company's activities to take place at the Humboldt University. Venture capital will be needed to provide further support for his commercial activities, and Dr. Sittinger is fairly confident that this will happen. His company currently has three employees.

Additional Information

Dr. Sittinger mentioned that there are three types of Lyme disease in Germany caused by three different bacteria. This disease is a major cause of arthritis in Germany and is transmitted by deer ticks.

Skin Tissue Engineering

Drs. Seifert and Kage both reported on their research activities in skin tissue engineering. For purposes of placing these research activities in the context of the eight chapters of this WTEC report, these research activities can be summarized as follows.

Biomaterials

Dr. Kage reported on the use of a silicone polymer with a hydroxyapatite coating that leads to a confluent growth of keratinocytes. In a review of the first draft of this site report, Dr. Kage commented that the material used for transplantation has certain characteristics such as high elasticity, degradability, and high transparency. It is different from the biocompatible silicone polymer coated with hydroxyapatite described for confluent cell growth.

Cells

The major effort of the work presented by both investigators focused on the cells used to tissue-engineer their skin material. Dr. Seifert spent a lot of time discussing the reduction of immunogenicity of human keratinocytes by gene allogeneic therapeutic methods. The goal is to make a skin equivalent with genetically modified cells, since autologous sources of skin are in short supply.

Dr. Kage prefers to use autologous derived cells. He has been able to grow autologous keratinocytes obtained by skin biopsies on his special membrane material (a silicone polymer coated with hydroxyapatite), and transplant this skin onto children that have had keloids resected for improving joint mobility. The transplanted skin had very high capillarity and showed signs of sensibility and of the incorporation of melanocyte cells.

Engineering Design Efforts

The reported results were primarily found in the laboratory and involved very little engineering effort.

Legal and Regulatory Issues

It was not clear whether Organogenesis's "Apligraf" product has been approved for use in Germany. There are no approved German-made tissue-engineered skin products at this time.

Dr. Kage has formed his own company (MeGaTec) to make his special membrane material that is a very good cell adhesive.

Dr. Seifert mentioned that human fetal stem cells are not allowed to be generated for a patent application in Germany.

REFERENCES

German Heart Institute. Biotechnology—science and technology for the next millenium. (brochure).

_____ Glycobiotechnologie—new perspectives for diagnostics, molecular medicine and nutrition. (brochure).

_____ Big/ tib. (brochure).

_____ Tissue engineering—key technology for biomedicine of the 21st century. InteressenGemeinschaft. (brochure)

_____ 2000. Symposium on therapeutic applications of human stem and precursor cells. Hannover Medical School. (brochure).

_____ Biotechnology in Berlin-Brandenburg. Land Brandenburg. (brochure).

_____ The medical technology business in Berlin-Brandenburg. Land Brandenburg. (brochure).

_____ Tissue engineering in Berlin-Brandenburg. Land Brandenburg. (brochure).

_____ 2000. Expo2000 Hannover. *BioTop*. (brochure) 1-20.

_____ 2000. Berlin-Brandenberg. (handout). 4-40.

Site: **ERA Consulting (UK), Ltd.**
10-16 Tiller Road
Docklands
London E14 8PX
England
(The meeting took place at the ERA office in Germany.)

Date: 20 July 2000

WTEC Attendees: D. Smith (report author)

Host: Christopher J. Holloway, PhD
Director of Regulatory Affairs and CSO

BACKGROUND

ERA Consulting is a regulatory affairs and product development company with offices in Germany, Great Britain, and the United States. It consults with medical product developers, especially pharmaceutical companies, to develop regulatory approval strategies and to act with those businesses in their engagement with governmental agencies regulating the clinical testing and use of medical products. Dr. Holloway has particular experience with recent developments within the EU and its member states to address the regulatory status of medical products incorporating living human tissues. ERA Consulting does not engage in coordinating clinical trials for its clients in order that it shall not benefit financially from the strategic advice it offers.

PURPOSE OF MEETING

When added to his extensive knowledge of the regulation of medical devices and pharmaceuticals worldwide, Dr. Holloway's experience with regard to human tissue products has given him an invaluable perspective for estimating the barriers to the clinical introduction of engineered tissues and for understanding the legal and regulatory issues.

DISCUSSION

See *EU Regulation of Medical Devices and Medicinal Products* and the site report for the European Agency for the Evaluation of Medicinal Products (EMEA) for a more comprehensive consideration of the treatment of engineered tissue products within the EU and among its member states.

Dr. Holloway outlined the present approaches of the EU member states to the regulation of human tissue products. A number of states, notably Spain and Portugal, do not presently regulate these products; Switzerland and France classify these products as "transplants"; Germany and Sweden have read the EU directives to classify these products as "medicinal products" by default; the United Kingdom has not made a final determination, but appears likely to treat these as "medicinal products" as well.

A number of reasons help to explain the disparity of classification decisions among these countries. A certain classification may allow for better utilization of existing regulatory resources (as where a national regulatory body's present scope of authority is out of proportion with its present manpower). Further, anticipating that a common classification decision may eventually emerge from the EU, national regulatory bodies may be reluctant to invest in establishing a process of analysis that may prove to be inconsistent with the common classification scheme. Dr. Holloway noted, though, that a few member states have elected to implement a regulatory process for human tissue products, presuming—from their interpretation of the plain meaning of Directive 65/65/EEC—the common classification of these products as "medicinal products." He believes that this presumptive classification will be officially adopted by EMEA in due course; in fact, he has already observed informal endorsement of the classification of human tissue products as "medicinal products" in

EMEA discussions regarding particular products. He added, though, that legal counsel in EMEA and Enterprise DG can exert a substantially conservative influence on regulatory engagement with emerging technologies; recent EU court decisions criticizing overly broad interpretations of regulatory authority have induced caution and circumspection in the application of that authority to new products.

Dr. Holloway anticipates that certain member states, especially Germany, are likely to force the classification issue to preclude the introduction of unregulated tissue products through states that presently do not regulate manipulated human tissues as medical products subject to pre-market demonstration of safety and efficacy. The reality of the common market is that engineered tissues introduced in one state could be freely delivered to and used in another state.

In the event engineered tissue products are recognized as "medicinal products," the importance of "regulatory science" (i.e., a validated process of tissue characterization and product investigation leading to the development of engineered tissue products within defined quality and performance specifications) cannot be underestimated. Dr. Holloway is concerned that EMEA will be challenged to approve early generation engineered tissue products that have come about through forms of clinical experimentation that may have tolerated greater uncertainty in the mechanisms and external influences of cell growth and tissue organization. Likewise, classification may facilitate reimbursement, but the product sponsor must still demonstrate significant improvement in therapeutic benefit from approved products and procedures.

Dr. Holloway does not anticipate that EU regulatory authorities will balk at approving engineered tissue products because of the type or source of the tissue used. He noted that the EU regulatory process is structurally insulated from political forces (absent a well-organized, pan-European effort, similar to the opposition to the introduction of genetically modified foods).

Prior to pre-market review, other factors may influence the pace of product development. Dr. Holloway pointed out that clinical trials can be difficult—especially in Germany, where individual states retain substantial autonomy to establish GMP requirements. Member states regulate the conduct of clinical trials without EU oversight; certain states may require pre-submissions, but may not review the materials submitted absent a report of an adverse event (then open the file delivered by the sponsor to determine if the submission was adequate).

Site: **ETH Zurich**
ETHZ Institute for Biomedical Engineering
Moussonstrasse 18
Zurich CH 8044
Switzerland

Date: 20 July 2000

WTEC Attendees: H.P. Greisler (report author), D. Mooney, L. McIntire, N. Parenteau

Hosts:

Andreas Zisch,
ETHZ Institute for Biomedical Engineering (J.A. Hubbel's group)
ETH Zurich Institute for Biomedical Engineering
Moussonstr. 18, CH 8092
Zurich
Email: zisch@biomed.mat.ethz.ch

Gaudenz Danuser
ETHZ Laboratory for Biomechanics (Prof. E. St ssi group)
ETH Zurich, Laboratory for Biomechanics
Wagistr. 4, 8952 Schlieren
Email: danuser@biomech.mat.ethz.ch

Hans-Peter Merkle
ETHZ, ETHZ Pharmacy (Merkle Group)
ETH Zurich, Galenical Pharmacy
Institute of Pharmaceutical Sciences
Winterthurerstr. 190, CH-8057, Zurich
Email: hmerkle@pharma.ethz.ch

William Pralong
University Hospital (CHUV), Lausanne (P. Aebischer's group)
Lausanne University Medical School (CHUV)
Surgical Research Division and Gene Therapy Center
Pavillions 3 & 4, CH-1011 Lausanne
Email: William.Pralong@chuv.hospvd.ch

Kadija Schwach
University of Geneva (R. Gurny's group)
University of Geneva, Department of Pharmaceutics and Biopharmaceutics, Quai Ernest-Ansermet 30, Ch-1211, Geneva
Email: khadija,schwach@pharm.unige.ch

Marcus Textor
ETHZ Laboratory for Surface Science and Technology (N.D. Spencer's Group)
ETH Zurich
Laboratory for Surface Science and Technology,
Sonneggstr. 5, Ch-8092, Zurich
Email: textor@surface.mat.ethz.ch

BACKGROUND

The ETH research program in tissue engineering is a highly interrelated multi-investigator group located primarily at the ETH Zurich campus but including scientists at the University Hospital Lausanne, the University of Geneva, and other Swiss institutions. The labs function both independently and in collaboration with each other and with investigators internationally. The overall director is Professor Jeff Hubbell.

Primary funding opportunities in tissue engineering include the Swiss National Research Foundation (SNF), primarily for basic research. Additionally there are so-called "priority programs" for enabling technologies and precompetitive projects (ETH and SNF) as well as funding from the Commission for Technology and Innovation (CTI), which supports competitive projects with monies derived 50% from government and 50% from industry. The industrial partner receives rights to inventions derived from the work. Additional industry funding supports product-oriented projects. Interestingly, some such support may come from both the banking and the insurance industries.

Funding by the Swiss National Research Foundation supports individual investigator-initiated projects. It also has established national research programs (NRP), including one designated "Implants and Transplants." Swiss Priority Programs (SPP) include biotechnology and information programs. Scheduled to begin in 2001 is a program entitled National Competence Centers in Research. This will support a primary center that will develop an ongoing collaborating network throughout Switzerland and internationally.

The NRP Implants and Transplants program began in July 2000 with funding of SFr 15,000,000. The stated focus is to promote state-of-the-art, future-oriented research in implant and transplant technology; to transfer results of this research to therapeutic applications; to initiate projects dealing with legal, ethical, economic and/or psychosocial issues; to foster interdisciplinary projects to link up issues from the humanities and social sciences with biology and medicine; and to actively participate in the social debate on future developments of transplant and implant technology.

The Swiss Center for Biomaterials brings together expertise in chemistry and in biology with the vision to utilize molecular design techniques for development of novel biomaterials. Approaches include biomimetics, biomolecular assembly, cell-mediated degradation, *in situ* transformation, and passivation. Efforts address cell-based sensing and modulation of biological signals. Efforts focus on developing materials not dominated by established intellectual propriety with a goal to provide opportunity for new entrepreneurs and small businesses.

R&D ACTIVITIES

Biomaterials

Among the research groups focusing on biomaterials is the group of Professor Hubbell. Dr. Zisch described this group as comprising 20 full-time biologists, biochemists, polymer chemists, organic chemists, and chemical and electrical engineers.

An underlying concept is the advantage of proteolytic degradation of the produced matrix via cell-mediated degradation. The goal is to engineer biomaterials with extracellular matrix functional properties allowing the matrix to receive information from the cells and the cells to receive specific information from the matrix (e.g., growth factors). A major focus is on polymer hydrogels that are both bioactive and biodegradable and formed by liquid to solid conversion *in situ*. These may be subdivided into two categories: completely synthetic poly (peptide)/polyethylene glycol (PEG) copolymers and fibrin-based hydrogels.

The poly (peptide)/PEG copolymers include protease substrates, cell adhesion sites, and heparin binding sites. Examples of receptor binding sequences for adhesion and migration include CRGDSP and CYIGSR. The fibrin-based hydrogels incorporate a desired added protein, e.g., a growth factor, added along with Factor XIII to the fibrin, enabling protein incorporation into the cross-linked fibrin polymer. The addition of heparin prior to inclusion of the heparin-binding peptides prolongs the time course of bioavailability of the peptides.

The group of Professor Gurny at the University of Geneva also focuses on biomaterial development in the Department of Pharmaceutics and Biopharmaceutics. As explained by Dr. Schwach, the group consists of 50 faculty and staff and focuses on five areas of interest:

1. Biodegradable drug delivery systems
2. Parenteral biodegradable drug delivery systems

3. Oral drug delivery systems
4. Physics of solid state systems
5. Novel routes of administration

Examples of applications of interest include guided tissue augmentation for periodontology using bioresorbable membranes for growth factor delivery. Semi-solid low T_g poly (ortho ester) (POE), which is degraded by surface erosion and is hydrophobic, is used as the delivery vehicle. Another focus is on intraocular injection of bioerodible sustained release systems, again based on POE, for failure of glaucoma filtering surgery and for age-related macular degeneration.

Cells

The group at ETH Zurich under the direction of Professor Merkle has an interest in genetic engineering of dendritic cells to enhance antigen presentation. Dendritic cells are key in both humeral and cell-mediated immunity, and the group utilizes these cells to deliver DNA in microspheres phagocytized by the dendritic cells. In essence, DNA of interest is encapsulated in microspheres and phagocytosed by the cell. The intracellular phagosome is acted upon by lysosomal enzymes, the DNA escapes to the nucleus, and the transgene results in increased expressed of cell surface MHC Class I antigens.

The Lausanne group of Professor Aebischer also focuses on genetically engineered cells for peptide delivery in chronic systemic diseases including anemia and diabetes. Dr. Pralong provided an overview of this work, which is done in collaboration with groups at Brown University (Providence, RI), in Paris, and in Chicago. Detection of the transgene product has extended out to one year thus far. Both *ex vivo* and *in situ* gene transfer techniques are employed.

Another major focus for this group is the biohybrid artificial pancreas, using islets within hollow fiber membranes implanted via vascular interpositions.

Biomolecules

Utilizing novel polymer hydrogel delivery systems described above, the Hubbell group has a major focus on delivery of growth factors for angiogenesis. Efforts address induction of endothelial cell repopulation of both vascular graft and native vessel surfaces as well as revascularization of ischemic tissues. Dr. Zisch primarily addressed the use of VEGF. Studies have shown both covalent bonding of $VEGF_{121}$ and fibrin-heparin based incorporation of $VEGF_{165}$ to promote endothelial cell proliferation *in vitro* and capillary differentiation in a three-dimensional angiogenesis assay. Maximal activity occurs at concentrations of 1 μg/ml of fibrin gel. Increasing the relative concentration of heparin prolongs the VEGF bioactivity because relatively more release is dependent on heparinases and plasmin as opposed to passive diffusion. Early *in vivo* application of fibrin gels containing PDGF-AB on skin wounds of steroid-treated mice appear promising.

As described above in the section Biomaterials, the Hubbell group has a major focus on inclusion of cell-specific adhesion sequences, including those based on the RGD and the YGSIR sequences into the polymer hydrogel.

The Institute of Pharmaceutical Sciences of the Department of Applied Biosciences under the direction of Dr. Merkle has a strong interest in delivery of biomolecules for tissue-engineering applications. Current projects include delivery of IGF-1 from PLGA microspheres for induction of bone formation, work done in collaboration with the University Hospital, Children's Hospital, and School of Veterinary Medicine, all in Zurich. Using molecular biologic techniques, RT-PCR, etc., these researchers have addressed in detail cellular responses to the delivered IGF-1. Another major focus of this group is on nerve guidance conduits using hydrogels loaded with microspheres (PLGA) containing nerve growth factor (NGF)

The Hubbell group also addresses osteoinduction using hydrogel delivery of rh BMP-2.

Engineering Design

Dr. Textor detailed work in the research group of Professor Spencer in the laboratory for Surface Science and Technology at ETH Zurich. The thrusts includes tribology (conventional and nano), biomaterials (metals and polymers), and biosensors (optical wavelength). Surface-modification approaches include selective protein adsorption and resistance, critical to development of biosensors by elimination of nonspecific adsorption. Other approaches include biochemical functionalization, chemical patterning, and surface structuring (texture). Examples include photolithographic modifications of titanium. This group is dissecting the effects of topography vs. chemistry for cell interaction in collaboration with the University of Texas, San Antonio. Biological issues addressed include surface modifications for cell localization and effects on the cytoskeleton and on cell function. Major technical approaches include lithography, self-assembly techniques, and microcontact printing.

Using optical waveguides, this research group immobilizes receptor molecules with controlled orientation, primarily in two-dimensional patterns.

Cell-Based Sensors

As described above in the section Engineering Design, the Spencer group has a major focus on induction of surface resistance to nonspecific protein adsorption. These techniques are highly relevant to development of biosensors.

Information Technologies

Dr. Danuser at the Laboratory for Biomechanics has a very active program in quantitative microscopy to investigate dynamic mechanisms in cell-substrate and cell-cell adhesion. The mission of this BMMC group is to provide support for biologic hypotheses by electron microscopic and other photo-physicial techniques. Using sophisticated optical waveguide laser spectroscopic analyses, the group has produced real-time dynamic intracellular video imaging of cytoskeletal actin activity. These approaches combine with three-dimensional modeling to assess cytoskeletal rearrangement in response to known shear rates. Studies will correlate flow kinematics and cell shape with cell growth, retraction, and migration.

This group provides a powerful resource for all collaborative laboratories in the area of tissue engineering.

REGULATORY ISSUES

Intellectual property issues vary among the institutions, but in general, the patent is held by the institution and royalties are divided between the institution and the inventors according to established formulae.

ETH began a course on aspects of business establishment for scientists approximately six years ago and additionally provides contacts among the business community.

Research grant costs do not include overhead, with the exception of studies requested by industry in the form of contracts. As such, all funds are in the form of direct costs.

Faculty may function as industry staff, including ownership positions, to a maximum of 20% work effort.

Site: **European Agency for the Evaluation of Medicinal Products (EMEA)**
7 Westferry Circus
Canary Wharf
Docklands
London E14 4HB
England

Date: 17 July 2000

WTEC Attendee: D. Smith (Report Author)

Hosts: Dr. John Purves
Head of Biotechnology Sector

BACKGROUND

The EMEA was established in 1993 by EEC (now EU) Council Regulation No. 2309/93 to implement procedures to give effect to a single market for "medicinal products" among the member states. In conjunction with three directives adopted concurrently (Council Directives 93/39EEC, 93/40EEC, and 93/41EEC), the regulation authorized EMEA to manage a "centralized procedure" for a Community authorization to market medicinal products for either human or veterinary use. The directives also established a "mutual recognition procedure" for marketing authorization of medicinal products based upon the principle of mutual recognition of authorizations granted by national regulatory bodies. These procedures came into effect on January 1, 1995, with a three-year transition period until December 31, 1997. As of January 1, 1998, the independent authorization procedures of the member states are strictly limited to the initial phase of mutual recognition (i.e., granting marketing authorization by the "reference member state") and to medicinal products that are not marketed in more than one member state. Consequently, sponsors seeking marketing authorization for medicinal products throughout the EU are obliged to seek such approval through the centralized procedure administered by EMEA.

The concept of a "medicinal product" in Community legislation substantially predated the organization of EMEA. Council Directive 65/65EEC of January 26, 1965 defined the term to include:

> any substance or combination of substances presented for treating of preventing disease in human beings or animals.

[and]

> any substance or combination of substances which may be administered to human beings or animals with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in human beings or in animals is likewise considered a medicinal product.

A "substance" is further defined to include "[a]ny matter irrespective of origin which may be human . . . animal . . . vegetable . . . [or] chemical" (*Directive 65/65/EEC, Article 1)*. However, the directive also makes clear that its regulation of medicinal products (and, through amendments to the directive recognizing the authority of EMEA, the "centralized procedure") do not apply to such products "intended for research and development trials" (*Directive 65/65/EEC, Article 2*).

Sponsors of medical products derived through tissue engineering ("tissue-engineered products") have reported substantial inconsistency among the regulatory bodies of the member states regarding the classification of such products for purposes of determining the applicability of national or EU marketing authorization requirements. A determination that engineered tissue products are "medicinal products" subject to the centralized procedure for authorization administered by EMEA will substantially clarify and rationalize the process by which such products may be marketed throughout the Community.

The EMEA's Biotechnology Working Party has considered, among other things, safety issues in the delivery of human somatic cell therapies and a definition of a "cell therapy medicinal product" (see "Points to Consider on Human Somatic Cell Therapy," CPMP/BWP/41450/98 draft). This definition would consider engineered human tissues to be "medicinal products" within the meaning of Directive 65/65/EEC, provided the engineered tissue was the product of:

1. …an industrial manufacturing process carried out in dedicated facilities. The process encompasses expansion or more than minimal manipulation designed to alter the biological or physiological characteristics of the resulting cells, and
2. further to such manipulation, the resulting cell product is definable in terms of qualitative and quantitative composition including biological activity. (CPMP/BWP/41450/98 draft, page 3/9)

The Biotechnology Sector of EMEA is likely to have primary responsibility for considering the authorization of engineered tissue products in the event they are classifiable as "medicinal products."

DISCUSSION

Dr. Purves reviewed the responsibilities and operations of the Biotechnology Sector and considered how an engineered tissue product may become classified as a medicinal product subject to the centralized marketing authorization procedure.

He noted that EMEA is a small organization with a total staff of about 200 persons. EMEA is charged with coordinating and managing the evaluation of medicinal products pursuant to the centralized procedure put into effect in 1995. Dr. Purves observed that, to date, EMEA has not worked directly in evaluating any engineered tissues, although he thought it more likely that such tissues would be handled as devices.

Human tissues used for medical therapy range from organ transplants to cellular products. Transplantable organs are presently the prerogative of the member states, although Dr. Purves could anticipate that transportation or other issues could require consideration of EU legislation. He did not consider cellular products to be readily definable as "medicinal products." He acknowledged that his impression of this distinction between cellular products and medicinal products is predicated upon his sense that such cellular products are presently the result of modest manipulation of autologous tissues in the course of treating a fairly small patient population. Under these circumstances, the regulation of such cellular products is more likely to remain with the competent authorities of the member states.

EMEA is aligned with Enterprise DG (formerly DG III; the department of the European Commission primarily responsible for establishing and implementing rules promoting the Single Market for products). A unit of Enterprise DG oversees application of EU directives regulating marketing authorization of medical devices. Consequently, Dr. Purves anticipates that providing for engineered tissue products could require some reconsideration of the specific areas of responsibility of the units or agencies involved in regulating medical products.

Dr. Purves added, though, that a decision to accept an engineered tissue product as a "medicinal product" could occur in response to a petition from a sponsor of such a product. He explained that, to be successful, such a petition should probably stress the "industrial" nature of the fabrication process and the extent of manipulation of the human biological material to produce the engineered tissue product. Assuming an engineered tissue product could be established to be a "medicinal product," Dr. Purves was not aware of any EU rule that could limit the ability of EMEA to grant market authorization according to the type or source of tissue from which the product had been derived.

Site: **European Molecular Biology Laboratory (EMBL)**
Meyerhofstr. 1
69117 Heidelberg
Germany
Tel. +49 6221 387-0. Fax. +49 6221 387-306
http://www.embl-heidelberg.de/

Date: 20 July 2000

WTEC Attendees: J. West (report author), F. Heineken, W. Wagner

Hosts: Dr. Andres Kriete
Bio., University Clinic Giessen Aulweg 123
35585 Geissen
Tel.: +49-641-997-165; Email: andres.kriete@anatomie.med.uni-giessen.de

BACKGROUND

The EMBL is an international cooperative effort to foster molecular biology research that was modeled after the Cold Spring Harbor laboratory. The main facility is located in Heidelberg, Germany, with approximately 50 research groups at this site. Approximately 20 additional research groups are located at EMBL substations (for instance, at a synchrotron facility). Funding comes from all of the member countries. The WTEC team met with several groups, as summarized below. EMBL is actively working to encourage technology transfer activities and development of small companies. It currently has a small start-up incubator facility.

R&D GROUPS

Dr. Stelzer's Group

The research foci of this light microscopy development group include optical levitation and laser tweezers to manipulate cells and tissues (including embryos) and the development of a BioImage database. Our visit covered mainly the BioImage database. This project was funded by the European Commission and involves 8 different institutions. The goal is the development of a database to handle protein- to tissue-level imaging data (including electron microscopy, X-ray diffraction, scanning probe microscopies, light microscopy, and video microscopy). The database stores all imaging data as well as supplemental information such as links to related literature, specimen information, sample preparation, and instrumentation details. The intent is to store images associated with publications, and the investigators are seeking to license their program to a publishing company. There are currently only 33 entries in the database, as the focus has been on the development of the database structure. Content will be added upon commercialization. The database can be viewed at http://www.bioimage.org.

Institute for Molecular Medicine, Dr. Hentze

Molecular medicine is a new focus area at the EMBL, intended to foster research with direct applications to medicine. This institute includes a number of research groups at the EMBL. Many of these groups are working in functional genomics (most located at an EMBL substation, the European Bioinformatics Institute). Other research areas include proteomics, post-transcriptional control, and cell biology.

Dr. Ellenberg's Group

Dr. Ellenberg is studying mitosis in cells and embryos. He follows the events during mitosis via confocal microscopy. He is working with GFP (green fluorescent protein) fusion proteins of various nuclear envelope proteins. He uses cationic liposome transfection with inducible promoters in initial studies and homologous

recombination in embryonic stem cells for more detailed studies. Dr. Ellenberg collaborates with groups developing new confocal imaging techniques and new image processing techniques.

Advanced Light Microscopy Facility, Dr. Pepperkok

Much of the work done in this facility is done in collaboration with microscope manufacturers. Microscopes have been adapted with unconventional lasers to allow detection of multiple fluorophores. A real-time confocal microscopy system has been developed. This system utilizes a spinning disk with apertures of various sizes. Work is also done in 2-photon confocal microscopy, FRAP (fluorescence recovery after photobleaching), and FRET (fluorescent resonance energy transfer). Data from microscopes is sent directly to the server to be available for remote access.

R&D ACTIVITIES BY TOPIC

Cells

Activities include manipulation of live cells with laser tweezers and optical levitation, live cell imaging, and homologous recombination of genes into embryonic stem cells. There may be other relevant research that we did not have time to discuss.

Biomolecules

GFP fusion proteins are being used to study cellular events. There may be other research in this area that we did not see.

Informatics

Activities include functional genomics, proteomics, imaging, image processing, image databases.

Tech Transfer

There is an incubator facility on site.

REFERENCE

European Molecular Biology Laboratory. 1999. Scientific Programme 2001-2005. *European Molecular Biology Laboratory*.

Site: **German Cancer Research Center (DKFZ)**
Im Neuenheimer Feld 280
69120 Heidelberg
Germany

Date: 20 July 2000

WTEC Attendees: W. Wagner (Report author), F.G. Heineken, J. West

Hosts: Dr. Roland Eils, Department of Intelligent Bioinformatics Systems (iBioS)
Dr. Daniel Gerlich
Dr. Markus Gumbel
Dr. Andreas Wunder, European Molecular Biology Laboratory (EMBL).

BACKGROUND

The German Cancer Research Center (DKFZ) in Heidelberg houses the Department of Intelligent Bioinformatics Systems (iBioS) headed by Dr. Roland Eils. This department has significant research efforts in bioinformatics. In the imaging area, its researchers are developing software to handle multidimensional image systems (i.e. 3D and 4D), fully automatic imaging systems for the evaluation of dynamic processes in living cells, and clinical imaging for cancer diagnostics. In association with DNA chip technology being utilized at DKFZ, the group is interested in image analysis for determining expression ratios and data mining. This methodology would be applied to the development of a tumor database.

Dr. Daniel Gerlich described a project on the visualization of dynamic processes in cell biology where co-localization of chromatin and a nuclear envelope protein were visualized in a continuous time-space reconstruction. Other tools developed include object detection, object tracking, and image enhancement capacities. The group collaborates with institutes in the United States (e.g., Cold Spring Harbor) and other German groups where experimental data will benefit from its analytical capabilities.

Dr. Markus Gumbel is working on a model of C. *elegans* development. This project seeks to reproduce the cell division and migration leading to the formation of functional cell groups within this approximately 1000-cell organism. Such modeling might find application in tissue-engineered organ development and stem cell differentiation. At present, the model is empirical and fits data collected with microscopic image analysis.

The group at DKFZ is interested in the commercialization of its technology and has five patents (held by the University of Heidelberg). Microscope companies have licensed two of the patents, and the patent/license strategy is generally that which is being pursued. There is consideration, however, of the formation of a company for its data-mining activities. DKFZ is an independent institute associated with the University of Heidelberg. DKFZ has first rights to investigator intellectual property, whereas this is not currently the case at the University of Heidelberg. The DKFZ technology transfer office has 6 full-time and 2 part-time employees; it files on the order of 50 patents per year. Investigators can buy licenses to its patents from DKFZ for company formation, and this is encouraged by institute leadership. No limits are placed on investigator interest in a start-up company (it can be 100%), but investigators must work 40 hours per week at the institute and can only add approximately 8 hours per week in the business. This limit results from union limits on work week hours for state employees.

The government supports biotechnology start-up company formation with a program that awards funds matching venture capital investment. These awards are based on the evaluation of business plans submitted by the company and do not come with government financial interest in the company. Awards can be as large as $2.5-5 million.

A separate presentation from a researcher at DKFZ was given at the European Molecular Biology Laboratory (EMBL). Dr. Andreas Wunder described the use of albumin as a drug carrier for the treatment of cancer and rheumatoid arthritis. Dr. Wunder has observed that albumin accumulates in tumors, presumably due to increased permeability of the vasculature and low lymphatic drainage. He also indicated that tumor cells

endocytose albumin and digest it as a nitrogen source. For this reason he has chosen albumin as a drug carrier to preferentially deliver chemotherapeutic agents, such as methotrexate, to tumor cells. He has also covalently coupled fluorescent tags to albumin for tumor detection. This technology has been licensed and is in Phase II clinical trials. Dr. Wunder is now also investigating albumin-methotrexate conjugates for treatment of rheumatoid arthritis, hoping to achieve high methotrexate delivery to the inflamed and hyperproliferative synovial pannus.

R&D ACTIVITIES

Engineering Design

Computational techniques under development for the modeling of cell differentiation and migration may have the potential to evolve into useful tools for the design and optimization of tissue-engineered constructs from precursor cells.

Informatics

Imaging Tools. No imaging tools, only software tools, were discussed in our visit.

Remote Interactions. The group at DKFZ has extensive collaboration around the world. At present it appears that data exchange is performed by conventional means.

Image Data Analysis. The group is focused on this area and has produced tools for 3D and 4D image analysis

Genomics/Proteomics. There is an effort to develop data-mining techniques for the evaluation of a tumor database being assembled at DKFZ. This area was not discussed in detail in the WTEC visit.

Computational Biology/Chemistry. The modeling of *C. elegans* development would qualify as computational biology.

GOVERNMENT ACTIVITIES

Government restrictions on the number of hours devoted to outside business interests were discussed, as was the venture capital funding match available for start-up biotechnology companies.

Site: **German Heart Institute**
Augustenburger Platz 1
D-13353 Berlin, Germany

Date: 17 July 2000

WTEC Attendees: W. Wagner (report author), S. Gould, F.G. Heineken, J. West

Hosts: Dr. R. Sodian
Tel: 49/30/4593-2154; Fax: 49-/30/4593-2100; Email: Sodian@dhzb.de

BACKGROUND

The WTEC team did not have the opportunity to visit the German Heart Institute in Berlin, but did receive a presentation from Dr. Sodian on the development of tissue-engineered valved conduits. Dr. Sodian trained with Dr. John Mayer at Harvard University and also worked with Dr. Vacanti's group at MIT. Dr. Sodian described collaborative work with the Boston group wherein lamb carotid artery cells are seeded onto a poly(hydroxyalkanoate) matrix to form a pulmonary trileaflet valved conduit. In a recent development on this project, Dr. Sodian's group is applying rapid prototyping technology to shape the thermoplastic, biodegradable polymer matrix. The input for the rapid prototyping comes from CT scans of homografts and allows for the creation of more complex anatomical structures. A limiting factor at present is the resolution (about 1-2 mm) in this technique. This resolution leads to thickness problems in the fine leaflet structure. A company, Virtis, has been formed to pursue the technology, and intellectual property issues are being worked out with the Boston group.

R&D ACTIVITIES

Biomaterials

The materials used are obtained from the U.S. company Metabolix and are poly(hydroxyalkanoates). These materials are a second generation in this project, following the more standard poly(glycolic acid) and PLA/PGA copolymer.

Engineering Design

Computational methods associated with this project were not discussed. One could consider the use of rapid prototyping based upon CT images a type of engineering design.

Cells

Autotypic lamb carotid artery cells were used. Cells were expanded *in vitro* for 34 days prior to seeding on the matrix. No phenotype control or genetic manipulation was employed. The Boston group has studied various autologous vascular cell sources in previous publications.

Informatics

Imaging Tools. CT scans were utilized to form matrix structures using rapid prototyping.

Remote Interactions. The group collaborates with the group in Boston. The method of data exchange was not discussed.

Site: **Humboldt University of Berlin, Charité**
Campus Virchow-Klinikum, Dept. of Surgery
Dr. Gerlach Research Group
Hs.: 37, R.: 2.0102
Augustenburger Platz 1
13353 Berlin, Germany
http://www.cito.charite.net
(see also http://www.hybrid-organ.com/)
(Associated with Hybrid Organ GmbH)

Date: 18 July 2000

WTEC Attendees: J. West (Report author), S. Gould, F. Heineken, W. Wagner

Host: Dr. D. Kardassis
Tel: +49-30-450-559022; Fax: +49-30-450-559909
Email: dimitrios.kardassis@charite.de, joerg.gerlach@charite.de

BACKGROUND

This laboratory has developed a set of perfusion bioreactors that are in use as extracorporeal liver assist devices and that are under investigation for culture of hematopoietic stem cells. The bioreactor consists of a circular chamber with sets of capillary bundles (hollow fibers) spanning the device. Culture medium is perfused into the bioreactor via a set of bundles with the outflow closed; this forces the medium to cross the fiber and extracapillary space and enter another capillary bundle for outflow. Cells (parenchymal and non-parenchymal co-culture) are cultured in the extracapillary spaces. Cells have been cultured in this system for up to 8 weeks. Fourteen patients in fulminant or acute-on-chronic hepatic failure have been treated so far. The first eight patients were bridged to transplant with a device containing porcine cells (average treatment time 27.4 hr., range 8-46 hr.). Due to concerns about regulatory issues as the investigators move towards multicenter trials, the investigators chose to use human cells (obtained from discarded organs) and to avoid use of any animal proteins during culture for devices in the subsequent patients. Each bioreactor is generally loaded with 300-500 g of hepatocytes. Only one device is needed to treat a patient (many systems require several devices in series), and the device is used continuously. The bioreactor is housed in a portable incubator that can be transported from the laboratory to the patient's bedside. Currently, this therapy is subject to no federal regulation. It has been able to move into the clinic with hospital committee approval. The investigators have also performed clinical applications in Spain.

An additional aim is to develop a membrane-based culture model for reconstructed epidermal/dermal bi-layers using autologous cells for skin transplantation. Another topic is the culture of hepatic adult stem cells and the clinical utilization of bone marrow adult stem cell plasticity for liver disease therapy. Further research topics of this group include *in vitro* models to investigate the effects of hypothermia, hypoxia, and reoxygenation in cell cultures, as well as improvement of preservation concepts for clinical liver transplantation.

Hybrid Organ GmbH was created as a spin-off company to commercialize the devices. Bioreactors are now being manufactured there in a GMP facility. This company is located at the Tempelhof Airport, which will facilitate transport of viable cell-containing devices to distant sites. This laboratory was impressive for its smooth transition of technology and knowledge from the basic laboratory into the clinic and industry.

R&D ACTIVITIES

Biomaterials

There is no research in this area. The laboratory uses only off-the-shelf materials (PU housing, PES hollow fibers).

Cells

This lab had used porcine, but was using human cells at the time of the WTEC visit. There is a co-culture system. Basic research was being conducted in hematopoietic stem cells and skin cells.

Biomolecules

Human serum is used at start of culture only. There is some basic research on effects of different growth factors on hematopoietic stem cells and skin cells.

Non-medical

The bioreactor device is also used as a model liver for testing drug toxicity as an alternative to animal testing.

Engineering Design

The visiting WTEC panel noted that, though an elegant perfusion bioreactor was developed, no engineers have been involved in this work. Hosts commented later that they have had help of engineers (e.g., for the bioreactor housing development and its electronic control).

REGULATORY ISSUES

The devices were unregulated, and the laboratory was seeking multinational approval.

REFERENCES

Gerlach, J.C. 1996. Development of a hybrid liver support system: A review. *Int-J-Artif-Organs*. Nov. 19 (11):645-54.

Gerlach, J.C. 1997. Long-term liver cell cultures in bioreactors and possible application for liver support. *Cell-Biol-Toxicol*. Jul. 13 (4-5):349-55.

Site: **Biomaterial and Tissue Repair Laboratory (INSERM)**
Université Bordeaux 2
146, rue Leo-Saignat
33076 Bordeaux cedex, France

Date: 19 July 2000

WTEC Attendees: D. Mooney (report author), H. Greisler, L. McIntyre

Hosts: Charles Baquey, Directeur
Tel: +33-5757-1483; Fax: +33-5690-0517; Email: u443@bordeaux.inserm.fr
Laurence Bordenave
Joelle Amedee

SUMMARY

Dr. Baquey and his associates presented an overview of the tissue-engineering research in their laboratories, which focuses on biomaterials issues. There was significant discussion on funding opportunities and support for tissue engineering within France and the EU. The basic philosophy underlying this group's approach to tissue engineering is that understanding the basic processes by which biomaterials can be used to induce specific cell functions will translate to new regenerative strategies. The focus is on autologous cell therapies, as a hospital-based service.

ORGANIZATION OF CENTER/FUNDING OPPORTUNITIES

The Biomaterial and Tissue Repair Laboratory

- Approximately 16 full-time permanent staff, 5 post-doctoral fellows, 11 doctoral researchers, and 5 pre-doctoral researchers. The budget is approximately one million dollars per year.

Complements to the Lab

- Material Science and Technology program (significant synthesis expertise)
- Laser science program
- Establissment Francais du Sang (Centre Aquitane-Limousin): expertise in cell sorting, phenotyping, tissue banking, and genetic modification
- School for Engineers in Biomolecular Technology

Complementary Facilities (not yet functional at time of WTEC visit)

- Platform for functional genomics
- Platform for functional and metabolic MRI
- Pilot unit for production of innovative diagnostic or therapeutics methods or devices. The WTEC team's understanding is that this unit will in essence be an incubator for start-up methods. It is being established within the university with government funding and will have space for 15 groups at full capacity. There will be a total of 3 such facilities funded by the government. A major goal of the facility in Bordeaux will be developing pilot-scale processes for materials processing and device manufacture.

FUNDING OPPORTUNITIES IN FRANCE AND EU FOR TISSUE ENGINEERING

Recurrent

- INSERM (250 labs); funded 4 years with 2 renewals—after 12 years go back into general competition
- CNRS
- University funding
- CEA (atomic energy commission)

Special Programs

- Cell biomechanics (INSERM)—to finish soon after the WTEC visit
- Cell-biomaterial adhesion (CNRS/INSERM)—began in 1999
- Tissue Engineering (INSERM/CNRS)—funding was to start soon after the WTEC visit; 10-12 programs were awarded for 3 year periods at FF 200,000-300,000 /year (no salary).

Regional councils will fund equipment.

Support for R&D Involving Companies and Academic Labs

- EU has support for projects that fit within the framework of official projects. Two that are relevant to tissue engineering are Quality of Life, which had specific targets for bioactive material development, and Competitive and Sustainable Growth, which had targets for cell-transplantation therapies. In addition, there have been programs for international collaborations termed "Bio 1: bone substitutes," "Bio 2: Bioartificial organs and tissues," and "5th PCRDT: Biomechanical interactions in tissue engineering and surgical repair."
- Regional level funding is available, both alone and jointly with federal funding.
- A national agency for applications of research output (ANVAR) funds projects near market.

R&D ACTIVITIES

Biomaterials

The overall objectives of this laboratory are to understand the phenomena that control biomaterial integration and understand cell adhesion, inflammation, and angiogenesis. The tools utilized by this group include the use of model materials used to study cell phenotype *in vitro* and *in vivo*. This group's researchers are interested in what they term bioactive materials (synthetic materials plus bioactive agents or synthetic materials with biomimetic properties) and bioartificial materials (synthetic materials plus a biological component or combinations of biological macromolecules). They are also performing basic studies on the strength of adhesion utilizing peptides coupled to the tips of AFM probes.

Naturally derived materials. This group utilizes combinations of naturally derived materials (e.g., collagen plus elastin) as biomaterials. It is developing cellulose scaffolds with controlled porosity. In addition, it has developed radiation grafting techniques to couple biological macromolecules to the surfaces of synthetic polymers in order to infer bioactivity.

Synthetic materials. The group is not involved in the synthesis of new materials, but is actively involved in the use and modification of existing materials. Cell adhesion peptides are being coupled to titanium alloys as a component of the group's bone work (see below), and it is also working with PDVF vascular grafts. In addition, the lab will be one center of a multicenter clinical trial using autologous endothelial cells to coat PTFE vascular grafts.

Biomimetic materials. A variety of synthetic peptides are being utilized to coat materials in an attempt to regulate the phenotype of adherent cells and promote tissue regeneration. The lab is using both RGD-containing peptides, as well as purine analogs, and studying the role of peptide conformation (e.g., cyclic

versus linear) on the resultant cell response. Many of the studies involve adsorbed peptides, but methods for covalent coupling are available in-house.

Ceramic materials. A variety of materials, including hydroxyapatite, tricalcium phosphate, and coral, are being utilized in the lab's bone work.

Biomolecules

The focus in this group is the development of biomaterials that induce tissue formation, not the delivery of diffusible molecules (e.g., Manchester group). This work is described above.

Cells

The main focus is autologous cells that are expanded in the hospital setting. The model systems used in basic studies are human. This group will be part of multicenter clinical trial using human endothelial cells (vein-derived) seeded onto PTFE vascular grafts.

An important issue for this group is the effect of co-culture on cell phenotype and gene expression. In specific, it is utilizing co-cultures of endothelial cells and osteoprogenitor cells (stromal cells). Researchers here have demonstrated direct contact between these cells, via gap junctions, which influences phenotype dramatically.

Engineering Design

Transport issues. Researchers at this lab are co-culturing endothelial cells and osteoprogenitor cells with the ultimate goal of co-transplantation to enhance vascularization. Currently, they are transplanting cell-polymer scaffolds around arteries to enhance nutrient transport and demonstrating improved bone formation. They do not appear to be heavily involved in bioreactor studies, but they recognize this will be important in the future for their vision of hospital-based expansion of autologous cells.

Biomechanics. Flow systems are being utilized to study endothelial cell proliferation in the context of the vascular graft work. In addition, these researcjers have developed a system to radiolabel cells in the vascular grafts and monitor denudement in real time as a function of flow conditions. This model system is an excellent example of the intersection of various engineering disciplines required to pursue tissue-engineering goals.

Site:	**Imperial College of Science, Technology and Medicine** **School of Medicine, Tissue Engineering Centre** **Chelsea and Westminister Hospital, 3rd Floor** **369 Fulham Road** **London SW10, UK**
Date:	18 July 2000
WTEC Attendees:	L. McIntire (Report author), D. Mooney, N. Parenteau
Hosts:	Professor Julia M. Polak, MD., DSc, FRCPath. Tel: 44 020 8383-3231; Fax: 44 020 8743-5362; Email: julia.polak@ic.ac.uk Drs. Buttery and Bishop (Professor Hughes), Dr. Amis, Dr. Wallace, Dr. McCarthy, Dr. Reichert

BACKGROUND

The Tissue Engineering Centre at Imperial College received a 3-year development grant from the Medical Research Council in 1998. Additional funds to support the Centre have come from the EPSRC, Imperial College, and industry. The strengths of the Centre include molecular and cell biology and some aspects of biomaterials (particularly ceramic based bioglass). The Medical School at Imperial College combines several formerly independent medical schools and hospitals, including Chelsea and Westminister, Hammersmith, St. Mary's, and Charing Cross. In addition, the cardiovascular group at the National Heart and Lung Institute (NHLI) also participate in the Centre.

The Administrative Academic Board of the Centre is chaired by Professor Julia Polak. The Centre has six "thrust areas," listed below with the name of the person and institution heading each area:

1. Embryonic Stem Cells (ES cells), Prof. Polak of Chelsea and Westminister
2. Immunotolerance, Prof. Lechler at Hammersmith Hospital
3. Bioengineering (imaging and minimally invasive surgery), Prof. Kitney at South Kensington
4. Cardiovascular, Prof. Yacoub at NHLI and Harefield Hospital
5. Hard Tissue, Prof. Hughes at Charing Cross
6. Biomaterials, Prof. Hench at South Kensington

The entire Centre meets every 6-8 weeks to present progress. As the Centre has grown, this meeting has become somewhat unwieldy. Individual research groups meet much more frequently.

R&D ACTIVITIES

At Chelsea and Westminister, the panel met with Prof. Polak and Drs. Buttery and Bishop. Dr. Buttery presented the Centre's work on bone tissue engineering. The program can be viewed as having three major components.

Cell Source

(a) primary cells

(b) stem cells (mesenchymal or embryonic)

Scaffold Design

- inert or bioreactive materials

Implantation

(a) integration with native tissue

(b) immunological component (including gene therapy applications)

The goal is to control cell behavior and phenotypic expression, optimizing culture conditions to control cell growth and differentiation. The group is moving towards the use of defined media and development of bioreactors to control physical force loading and oxygen tension. There is also an interest in the space bioreactors and the effect of gravity on tissue culture and structure formation.

The emphasis is on the use of embryonic stem cells, as others are further along using mesenchymal stem cells. Another approach is being investigated using primary cells transfected with telomerase. There are potential problems of phenotypic stability, malignant transformation, and transfection efficiency with this approach.

A major effort is to understand the mechanistic pathways involved in osteogenic differentiation of embryotic stem (ES) cells. The timing of signals induced by phosphate, ascorbic acid, and dexamethasone are of special interest. Microarray technology will be employed in fellow gene expression profiles during differentiation. Growth of osteogenic cells on bioglass is being investigated. Bioglass induces cell cycle activity in human primary osteoblasts, but also induces apoptosis. Bioglass products have inductive effects on ES cell gene expression. The roles of insulin, like growth factor (IGF) and NO, in this process are being pursued.

Dr. Bishop presented her initial work in lung tissue engineering. Using murine ES cells, she allows growth of embryonic bodies and then identifies "lung buds." These cells are then subcultured with the goal of developing an alveolar culture, including optimizing media requirements. Again, understanding the mechanisms controlling gene expression profile during development is an overreaching goal. The process of partial differentiation to endoderm and then going back is an interesting approach. Dr. Bishop is in the first year of what she estimates to be a nine-year project.

In the afternoon the panel moved to Charing Cross Hospital and to a visit with Professor Hughes' associates, involved in more applied bone and ligament research, centered in the Department of Orthopaedic Surgery.

Professor Amis, who is in the Mechanical Engineering Department at Imperial, reviewed his extensive work on the use of polyesters for ligant repair. These materials had problems in human applications due to bone tunnels into attachment points. He is now thinking of using resorbable fibers—composed of bioglass powder with PLA/PGA co-polymers. One needs at least 25% bioglass powder for bioactivity.

Dr. Amis also has a rapid prototyping apparatus that may be useful for some orthopaedic tissue-engineering applications.

Dr. Wallace, a surgeon with a PhD in biomechanics and who has been at Imperial College for about 1 year, discussed his work on shoulder ligants. He believes these ligaments may have a possible neural role as an *in vivo* "strain gauge" in addition to having a mechanical function. His main interest is in extracellular matrix production in response to injury. To monitor functional gene expression, he works with the Kennedy Institute, which has significant expertise in proteomics (2D gel-electrophoresis). He is interested in the role of mechanical environment in gene expression and has identified a novel protein in cartilage from arthritic patients. While at Calgary, Dr. Wallace was involved in interesting studies employing anti-sense to decorin to examine the role of that protein in a rabbit scar model. His group demonstrated that inhibiting decorin expression led to larger filament function. Dr. Wallace has brought this technology to Imperial College.

Dr. McCarthy discussed his work on the role of fluid convection within the bone structure on mass transfer to osteocytes. His group has demonstrated significant fluid flow and is now involved in understanding detailed sources of this flow and its importance to bone homeostasis.

Dr. Reichert discussed her work on sheep tibia reaming to selectively reduce blood flow.

R&D ACTIVITIES BY TOPIC

Biomaterials

Bioglass is the primary biomaterial in the Centre, sometimes in combination with PLGA copolymers.

Biomolecules

Work is ongoing on the role of IGF and NO in ES cell osteogenic induction. Antisense technology is used to control production of extracellular matrix.

Cells

Embryonic stem cells (primarily murine) are used, as well as telomerase-transfected primary osteoblasts.

Engineering Aspects

Work focuses on understanding the importance of mechanical forces in tissue repair and development and on understanding the possible importance of fluid convection in bone tissue mass transfer. Future interaction is possible with Imperial College Chemical Engineering Department in development of appropriate bioreactors (still nascent).

Informatics

Microarray technology is used for gene expression profiles and proteomics for functional gene expression.

REGULATORY ISSUES

Imperial College scientists are apparently quite experienced in intellectual property regulations. They have developed some incubation facilities but probably are not as far along as Manchester. Human tissue accessibility now requires extensive informed consent. There is an increasing awareness in the UK of possible liability issues. Industry partners usually pay patent costs after sufficient preliminary data are accumulated.

Site: **Imperial College of Science, Technology and Medicine**
Department of Chemical Engineering and Chemical Technology
Prince Consort Road
London SW7 2BY
UK

Date: 19 July 2000

WTEC Attendees: N.L. Parenteau (report author)

Hosts: Dr. Athanasios Mantalaris, Chemical Engineering and Chemical Technology Dept.
Tel.: +44-020-7594-5601; Fax: +44-020-7594-5604;
Email: a.mantalaris@ic.ac.uk

BACKGROUND:

The Department of Chemical Engineering and Chemical Technology at Imperial College of Science, Technology and Medicine is one of the leading chemical engineering departments in the UK. While active in biotechnology research in the areas of fermentation, wastewater treatment, and bioreactor design, the department has not been involved in tissue-engineering applications. Dr. Mantalaris joined the department in March 2000. He comes from Dr. David Wu's laboratory in the Chemical Engineering Department at the University of Rochester. Dr. Mantalaris is originally from Greece.

He is preparing to continue the work on *in vitro* hematopoiesis systems started with Dr. Wu and to expand into additional areas of tissue engineering through his relationship with other faculty in the Imperial College Centre for Tissue Engineering. He hopes to stimulate increased involvement of other department faculty with expertise in the areas of bioreactor design, polymers, and modeling to address research problems in tissue engineering applications.

GROUP OVERVIEW

At the time of the WTEC visit, Dr. Mantalaris was still in the process of setting up his lab and establishing collaborations within the Centre for Tissue Engineering at Imperial College. His research on hematopoiesis involves the use of packed bed bioreactors, which serve as niches for hematopoietic cells. This system allows the development of critical cell-cell interactions, resulting in the enhancement of hematopoiesis over the traditional flask (Dexter) culture system. His research directions will be in the areas of directed three-dimensional hematopoiesis, gene function analysis, establishing embryonal hematopoiesis models, and modeling of mammalian cell culture systems.

Regarding directed three-dimensional hematopoiesis, Dr. Mantalaris is planning to construct a "virtual model" of the bone marrow structure that will be utilized to construct a "topographically accurate" scaffold, using microetching, which will foster specific lineage development. This will be enabled by the spatial binding of growth factors in these niches, for example the binding of VEGF to "sinusoidal-like" spaces. He also proposed the concept of "personalized bioreactors" to meet specific patient needs.

Dr. Mantalaris also described a serendipitous finding that he hopes to pursue at Imperial College. Embryonal teratocarcinoma cells, with a consistent malignant phenotype when grown on a PLGA scaffold, took on an endothelial cell phenotype where some cells showed differentiation and maturation into hemoblasts, immature red blood cells. The yolk sac is the site of earliest hematopoiesis, and Dr. Mantalaris believes that the embryonal teratocarcinoma cells are recapitulating some of this developmental lineage within the scaffold. He plans to develop this system in a microgravity bioreactor to simulate the neutral buoyancy in utero.

PARADIGM SHIFTS

Paradigm shifts seen in this visit are (1) the introduction of tissue engineering in a more traditional chemical engineering department, and (2) the collaborative and multifaceted work Dr. Mantalaris is attempting. He commented that unlike in the United States, Imperial fosters and supports collaborative efforts by junior faculty. There is less pressure to prove oneself as an individual researcher; it is possible to show value and individual accomplishment through collaborations. He also stated that £3 million had been set aside for start-up companies and that start-ups were seen forming at the rate of about one per month. Imperial does appear to provide guidance. He believed that the culture was good for fostering progress and that although they were still more cautious about change than in the United States, there was no longer the need to go to the United States to get ahead.

Site: **University of Glasgow**
Institute of Biomedical and Life Sciences
West Medical Building
G12 8QQ Glasgow, Scotland
UK
http://www.gla.ac.uk/Acad/IBLS/

Date: 21 July 2000

WTEC Attendees: M. Mrksich (Report author)

Host: Professor Adam Curtis
Tel: (+44) 0141-330 3524; Fax: (+44) 0141-330 4758

SUMMARY

The Institute of Biomedical and Life Sciences at the University of Glasgow is recognized as a center of excellence for studies of cell-substrate interactions. A long-standing collaborative effort between Professor Curtis, a cell biologist, and Professor Wilkinson, an electrical engineer, has pioneered sophisticated investigations of the influence of substrate topography on cell behavior. These researchers have recently joined with bioengineers and chemists at Strathclyde University to establish the Centre for Cell Engineering. The Centre's charge is to coordinate both fundamental and applied research in the broad areas of cell engineering and tissue repair. The Centre has established collaborations with both large and small companies, and with leading international groups in Germany (Dr. Offenhausser, Max Planck Institute) and Japan (Prof. T. Okano, Tokyo Women's Medical University).

Prof. Curtis reports that funding of basic research in this area has traditionally been inadequate, but that support from the European Community has proven significant in recent years. The commercialization of university-derived technologies is still not efficient and in many cases not pursued aggressively.

SUMMARIES OF TECHNICAL PROJECTS

Influences of Substrate Topography on Cell Adhesion

The researchers in this group were the first to combine sophisticated methods in microfabrication with a strong position in cell biology to address the adhesion and migration of cells on substrates having topographical features at the micron length scales. Recent work has extended these studies with the development of embossing methods to investigate the influence of substrates having defined topography at the nanoscale. Key achievements have been the identification of topographical patterns that both promote and inhibit cell attachment. Overall, this work has added significantly to a fundamental understanding of cell adhesion and cell-materials interactions.

Formation and Properties of Neural Networks

The researchers in this group have been the initiators of programs to pattern neurons into two-dimensional networks. They have a leading position in combining cell biology with the patterning and microelectronics for fundamental studies of electrical activities in networked neuronal cultures. The team has not yet emphasized practical outcomes of this work.

Biosensors

Professor Jon Cooper of the Department of Electrical Engineering has led efforts to develop biosensor technologies. The designs are based on microelectrode arrays that are used to interrogate samples by way of cell or protein-based assays. In one example, the enzyme-coupled electrodes are used to detect the release of small molecules from adherent cells. Related work is developing five electrode systems to simultaneously

stimulate cells and monitor bioactivities. These researchers have made important contributions to engineering the packages for the cell compartments, including the design of a microthermocouple to measure temperature changes in the cells. Other work is fashioning methods for deposition of silica to install waveguides for integrating fluorescence-based detection into the sensors and for bonding glasses to encapsulate channels used in microfluidics. They have developed a number of methods based on photopatterning and are currently developing methods to pattern microstructured three-dimensional structures.

The research effort at Glasgow has been very strong in fundamental studies and is now increasing the focus on applications centered on cell/materials engineering. The team has an excellent position in demonstrating collaboration between biologists and engineers (but has not yet integrated state-of-the-art surface chemistry). They are well positioned to develop and demonstrate practical technologies based on integrating cells with electronics and materials. The underdeveloped models for technology transfer will remain obstacles to realizing commercial successes.

Site: **IsoTis (technical report)**
Prof. Bronkhorstlaan 10
3723 MB Bilthoven
Netherlands

Date: 21 July 2000

WTEC Attendees: J. West (Report author), S. Gould, F.G. Heineken, W. Wagner

Hosts: Dr. Jens Riesle
Tel: 31-(0)30 2295229; Fax: 31-(0)30 2280255; Email: Jens.riesle@isotis.com

BACKGROUND

This company was founded in 1996 and currently has over 100 employees, 50 in R&D activities. The company holds more than 75 patents. The company has projects in orthopedic devices (one of which is FDA-approved) and tissue engineering (skin, bone, and cartilage). Some of the facilities at the company include a tissue-engineering clean room, a manufacturing and polymer synthesis clean room, and a regulatory department. Funding for this company (> $25 million) has come primarily from venture capital.

A major research focus in this company has been biomaterials development. One of its core materials, Polyactive, is a block copolymer of PEG and PBT. This polymer is synthesized in-house (10 kg/batch capacity). The polymers form flexible thermoplastics where mechanical properties, swelling, and drug release kinetics can be controlled by copolymer composition. These materials can be processed via compression, injection, or solution molding. This material has received U.S. FDA approval for use as a bone cement restrictor. This material is now being investigated for use as a tissue-engineering scaffold. Solution casting/salt leaching has been used to fabricate scaffolds with >90% porosity, and sintering has been used to create scaffolds with >50% porosity but superior connectivity of the pore structures. These Polyactive materials have also been investigated for controlled release of proteins, demonstrating zero order release for up to several months with little loss of activity.

We mainly discussed the cartilage tissue-engineering project. The approach in this project is to utilize an osteochondral implant where Polyactive seeded with autologous chondrocytes is used in the top layer with porous HA-TCP as the bottom layer (with ingrowth of cells from surrounding tissue to populate the osseous portion of the implant). The intent is to utilize autologous cells and serum. There is interest in using bone marrow-derived stem cells. Tissues are being cultured in spinner flasks or rotating wall bioreactors. There is interest in development of a new bioreactor to provide compressive stimuli. There is also interest in moving towards growth factor delivery and development of bioactive materials (immobilized growth factors, adhesive peptides, etc.). See the following IsoTis "regulatory report" concerning government and legal issues.

R&D Activities by Topic

- *Biomaterials*: polymer synthesis and characterization, ceramics, drug delivery, interest in bioactive/biomimetic materials
- *Cells*: autologous cells and serum, bone marrow-derived stem cells
- *Biomolecules*: growth factor delivery from scaffolds, attachment of bioactive factors to scaffolds
- *Engineering Design*: using currently available bioreactors; interested in developing a bioreactor to provide compressive stimuli

REFERENCE

IsoTis. 1999. Annual report. Catalog.

Site: **IsoTis (regulatory report)**
Prof. Bronkhorstlaan 10
3723 MB Bilthoven
The Netherlands

Date: 21 July 2000

WTEC Attendee: D. Smith (report author)

Host: Eliane Schutte, Head, Regulatory Affairs/Operations

BACKGROUND

As of the date of the WTEC site visit, IsoTis is a privately held medical device, biomaterials, and tissue engineering company. (Author's note: It has since completed its initial public offering of securities.) The company is commercializing a proprietary polymer for multiple applications with orthopedic products, and it is developing autologous bone and skin repair technologies. IsoTis was founded in 1996; it employs approximately 100 people at single facility in the Netherlands. The company concentrates on the development of autologous tissue products and therapies.

PURPOSE OF MEETING

Concurrent with a site visit to understand the company's technology platform and clinical trials programs, a separate meeting and discussion was held to consider relevant regulatory issues. Eliane Schutte, head of the Regulatory Affairs office, actively participates in tissue-engineering standards development efforts through ASTM (American Society for Testing and Materials) in North America and ISO/CEN (International Standards Organization / Committee for European Normalization) in Europe.

DISCUSSION

See *EU Regulation of Medical Devices and Medicinal Products* for a more comprehensive consideration of the treatment of engineered tissue products within the EU and among its member states.

Ms. Schutte explained the process of classification of medical products for regulatory oversight purposes to be a choice between designation as a "medicinal product" or as a "medical device." In terms of the nature or extent of that oversight, these classification alternatives represent a choice between a high degree of scrutiny possible through a substantially centralized procedure ("medicinal product") and a decentralized procedure utilizing a degree of self-regulation and oversight through nongovernmental notified bodies ("medical device").

In light of the definition of a "medical device" given in EU directives, this classification may not be available for the sponsor of an engineered tissue product, such that the sponsor would not have access to the notified body review process. Ms Schutte commented in correspondence with WTEC following this visit that, currently, tissue-engineered products do not fall under the category of "medicinal products." However, the sponsor's pursuit of a "medicinal product" classification would, if successful, invite a higher regulatory impact on the product development and marketing authorization process. This impact is balanced by the advantage of a uniform EU classification (especially in light of the divergent views of the member states over the regulatory status of engineered tissue products) and a reimbursement profile under the laws of the member states that is often more remunerative than what has been provided for medical devices.

Ms. Schutte noted the difficulties presented to her company by the differing classifications and degree of oversight employed by the regulatory bodies of the member states with respect to medical products incorporating human tissues. She understands that these products are not regulated in Italy, are classified as "transplants" (as opposed to medical products) in Spain and France, and are classifiable by default as

"medicinal products" in Sweden and Germany (although she has recently received an informal notification from the German national authority that autologous tissue products are not subject to regulatory oversight) . She is unsure how the United Kingdom will view these products, although she expects they are more likely to be classified as "medicinal products."

Ms. Schutte explained that the Netherlands does not regulate the use of human tissues in research and development, although legislation is under consideration to regulate the practices of tissue banks and other entities (including commercial industries) engaged in the transfer of tissues to humans.

Ms. Schutte noted growing interest within the tissue-engineering industry in Europe in the development of product standards. A working group has been organized to explore the establishment of a standards development process and evaluate the applicability of emerging ASTM standards to EU regulatory conditions.

Site: **Kirchhoff Institute of Physics (KIP)**
Institut für Angewandte Physik
Building Albert-Ueberle-Straße
Albert-Ueberle-Straße 3-5
69120 Heidelberg, Germany
http://www.kip.uni-heidelberg.de/index_e.shtml

Date: July 19, 2000

WTEC Attendees: F.G. Heineken (report author), S. Gould, B. Wagner, J. West

Hosts: Professor C. Cremer, Director
Tel.: +49-6221-54-9393; Fax: +49-6221-54-9262;
Email: Christoph.Cremer@kip.uni-heidelberg.de,
Dr. Peter Edelmann
Dr. Benno Albrecht

BACKGROUND

The visit to Heidelberg started with Dr. Andreas Kriete meeting us at the train station in Heidelberg. Dr. Kriete was our host for the entire day as we visited three sites in Heidelberg:

- European Molecular Biology Institute (EMBL)
- Kirchhoff Institute for Physics (KIP)
- German Cancer Research Center (Deutsche Krebs Forschung Zentrum (DKFZ))

VISIT TO KIP

This part of my report deals with the visit to the Kirchhoff Institute of Physics (KIP). See separate site reports by Jennifer West on the visit to EMBL and by Bill Wagner on the visit to DKFZ.

We met with Professor C. Cremer, who is Director of KIP, Dr. Peter Edelmann, who is a group leader at KIP, Dr. Benno Albrecht, who is on the staff at KIP, and two graduate students.

R&D ACTIVITIES

Cells

A lot of the discussion with KIP centered on cells and the quantitative analysis of the nuclear architecture of the cell. Computational modeling of the cell nucleus was discussed, as was the quantitative description of the access of transcription factors to the nucleus. Confocal microscopy with a normal resolution of 300 to 700 nanometers (nm) is the current capability at KIP for three-dimensional visualizing of chromosome labeling; the researchers wish to get this resolution down to the range of 35 to 50 nm. Dr. Edelmann described the KIP efforts to develop a spatially modulated illumination (SMI) microscope to carry out these higher-resolution measurements. Prior to leaving KIP, Dr. Benno Albrecht gave us a demonstration of the prototype instrument.

Biomolecules

Discussion of biomolecules focused on compounds for DNA labeling, for which Dr. Cremer has a U.S. patent.

Cell-Based Sensors

KIP researchers are primarily interested in sensing what is going on inside a cell, and that may be useful for the eventual use of cells as sensing devices, since methods are needed to detect signals from cells.

Engineering Design Aspects

KIP is doing the prototyping of its SMI instrument development, and there is some engineering involved in doing that. Its quantitative analysis of the cell nucleus also requires the use of some fundamental physical and engineering principles.

Informatics

Information processing is key to the KIP work on the quantitative analysis of the nuclear architecture. KIP appears to have state-of-the-art capabilities in information processing.

LEGAL AND REGULATORY ISSUES

KIP is savvy concerning protection of intellectual property and has a number of patents on its work.

GOVERNMENT ACTIVITIES AND INTERESTS

KIP is having difficulty raising funds to do its instrument development work. Its scientists see a great potential for the use of their capabilities for the detection of various genetic diseases, but they are having trouble convincing the German government of this potential. Dr. Cremer is thinking of applying for some NIH support. He is also interested in how the U.S. Government supports instrument development.

ADDITIONAL INFORMATION

Dr. Cremer spent approximately five years working at the Lawrence Livermore National Laboratory, and he has more recently developed a strong interest in medical physics. He has a brother (T. Cremer) who is a professor of genetics at the University of Munich.

Dr. Kriete is on the faculty at the University of Giessen and does collaborative work with a number of people in Heidelberg. The University of Giessen is about a one-hour drive north of Frankfurt.

Site:	**Manchester University** **School of Biological Sciences** **Stopford Building** **Oxford Road** **Manchester M13 9PT** **United Kingdom**
Date:	17 July 2000
WTEC Attendees:	D. Mooney
Host:	Dr. Timothy Hardingham, Professor of Biochemistry Tel: 44-(0)161 275 7513; Fax: 44-(0)161 275 5752
Attendees:	From both Manchester University and University of Liverpool, the Joint Tissue Engineering Centre: Timothy Hardingham (PI) John Hunt (Liverpool) Richard Black (Liverpool) Tony Freemont Mike Grant Cay Kielty Karl Kadler Robert Hawkins Ann Canfield Nick Rhodes

INTERDISCIPLINARY RESEARCH COLLABORATION (IRCOL) IN "TISSUE ENGINEERING: CELLULAR AND MOLECULAR APPROACHES"

The Joint Tissue Engineering Centre of Manchester and Liverpool Universities is a new center for tissue engineering, part of a British government program for interdisciplinary research collaboration (IRCOL). Funding was to start late in 2000. The discussion focused on the philosophy and structure of this center, not on research projects. The report is mainly focused on these aspects of the site.

Overall Philosophy/Vision (Timothy Hardingham)

Tissue engineering has largely utilized highly empirical approaches to date. The goal of this center is to bring a larger component of basic biological sciences, especially matrix biology, into the design of biomaterials and strategies for tissue engineering.

History of IRCOL

The UK Research Councils announced a competition for tissue engineering centers, and bids were due in November 1999. It was a requirement that applications be inter-institutional. Nineteen pre-proposals were submitted, of which four were chosen for full proposals. One (Manchester/Liverpool) was to be funded. The award of £9.7 million (including 43% overhead) over 6 years was given final approval after this WTEC visit, in September 2000. T. Hardingham (Manchester) will be director and David Williams (Liverpool) will be deputy director for the first 3 years, and they will switch positions for years 4-6. An interesting feature of this center is its proposal to maintain the IRCOL's ability to maintain focus by making training positions IRCOL fellowships. Trainees will be responsible mainly to the goals of the center.

Center Structure

The Joint Tissue Engineering Centre is focused around 3 clinical components, with 6 generic components (e.g., areas of technology) that can be mixed and matched to achieve the goals of each clinical goal. The clinical components are

1. Innovations in skin/wound healing (Mark Ferguson)
2. Cartilage/Invertebral disk (Tim Hardingham, Tony Freemont)
3. Vascular tissue engineering (Cay Kielty, Mike Walker)

The six generic components are

1. Control of cell phenotype and effect of fluid mechanics
2. Engineering 3D tissue structure
3. Biodegradable materials and bioactive surfaces
4. Tissue integration and angiogenic response
5. Inflammation and immunological issues
6. Development of gene transfer technologies

Of these, David Williams is heading up the first 5 areas, and Robert Hawkins is heading up the last.

There are a number of highly complementary activities already existing in Manchester/Liverpool:

- Matrix Biology Centre (Manchester). Funding for this center was renewed in 2000 by the Wellcome Trust for a second 5-year period. It is funded for £5 million over 5 years for cores, equipment, etc. Competitive research grants fund the specific research projects, and £30 million of funding is handled through the center. Many of the major players in this center are part of the tissue engineering center, and the matrix biology expertise of this center is a critical component of the center.
- Laboratory for Biomaterials and Tissue Engineering (Liverpool). A JIF (joint infrastructure fund) award provides £3.5 million to establish a new lab in these areas. This creates an infrastructure for the biomaterials component of the tissue engineering center.
- Clinical Research Centre (Manchester). This facility is one of five in this arena recently funded with £3-4 million by Wellcome Trust. It provides a means of conducting clinical research and trials and may provide a means to move tissue-engineering developments to the clinic.
- Manchester University has put together an impressive incubator facility, which is superior to any in the United States. This facility, which opened in 1999, is run by a private company owned by the university. This facility can accommodate up to 16 start-ups at a time, and had 6 tenants at the time of the site visit. An impressive feature of this facility is that it can provide support in numerous areas to the start-ups, including preparing business plans and providing financial operations (e.g., payroll) and intellectual property support. Michelle Cooper of the business development office indicated this facility will actively search out and acquire intellectual property complementary to that generated within the university in order to strengthen the start-up companies. Companies residing in the incubator facility may either be solely tenants or companies in which the university maintains equity.

R&D ACTIVITIES BY TOPIC

Biomaterials

Biomaterials work is likely the major strength of the Joint Tissue Engineering Centre. The matrix biology group at Manchester and biomaterials groups at Liverpool are both internationally recognized leaders in their areas. The integration of these two groups is potentially very powerful, and it should be expected they will make significant strides in biomimetic approaches to materials design.

The matrix biology group (Hardingham, Kielty, Canfield, Kadler) has expertise in virtually all areas of matrix biology, from genetics to macromolecular structure and assembly. William and colleagues have significant expertise in biocompatibility and work with a variety of naturally derived and synthetic materials. They are actively involved in molecular-level analysis of biocompatibility and surface modifications to infer specific types of bioactivity.

A recent example of the potential of this group to develop novel biomaterials can be found in the recent work of Dr. Karl Kadler, who is developing novel collagens and has expressed them in mice to date. Center scientists are working to produce such collagens in the milk of large animals, and these could be extremely valuable biomaterials.

Engineering Design

Biomechanics. Many of the clinical targets of this group have a large mechanical function, and the group recognizes these issues. Williams' group has been involved with experiments of fluid flow effects on cell phenotype and is initiating studies with flow bioreactors for its vascular work. However, other aspects of biomechanics are not yet being examined.

Transport. This group (mainly Williams) is performing some work utilizing bioreactors, but this does not appear to be a major focus. There is strong basic biology in Manchester in vascularization, and it is likely that collaborations with Liverpool will lead to applications of this work in a tissue-engineering context. Cryopreservation does not appear to be a focus at this time.

Cell Sourcing

At present, center groups focus on autologous and allogeneic cell research in a variety of animals models. There is not heavy involvment with cell transplantation approaches to tissue engineering.

Biomolecules

This group is very interested in regenerative medicine approaches based on biomolecules, and this will likely be a major focus of the Joint Tissue Engineering Centre. There is significant expertise in cytokine and growth factor biology in areas such as angiogenesis and wound healing. In addition, center scientists have associated expertise in gene therapy approaches they wish to apply to promote transient gene expression for regeneration approaches. The biomaterials and biomolecules group will make a powerful combination capable of moving basic biology discovery to delivery systems for biomolecules.

Site: **Max Planck Institute for Polymer Research**
Postfach 3148
55021 Mainz, Germany

Date: 19 July 2000

WTEC attendees: M. Mrksich (Report author)

Hosts: Professor Dr. Wolfgang Knoll
Tel: +49 (0) 6131 379 160; Fax: +49 (0) 6131 379 360
Email: knoll@mpip-mainz.mpg.de
Dr. Andreas Offenhausser
Tel: +49 (0) 6131 379 475; Fax: +49 (0) 6131 379 100
Email: offenhaeusser@mpip-mainz.mpg.de
http://www.mpip-mainz.mpg.de/~offenhae

SUMMARY

The Max Planck Institutes (MPI) are an important component of basic and applied science research structure in the German system. The institutes, which are generally not a part of the university system, each focus on an important area of science. The polymer research institute conducts research in the broad area of polymer science and technology. Six directors oversee a staff of 450 persons comprising 85 permanent staff scientists, 55 visiting scholar scientists, 150 graduate students, and 150 technical and administrative personnel. The institute has an annual budget of DM 35 million that includes DM 7.4 million of external funding. Overall, this institute is among the best in the level of infrastructure and technical staff.

The research portfolio is divided into projects that often involve close collaboration between multiple research groups. This organization is significant because it encourages extensive interaction and cross-disciplinary efforts. The group of investigators at the MPI for Polymer Research is very collegial, and the research that has emerged from this group clearly indicates extensive interaction between investigators with different backgrounds. The prospects for future work in the area of biomaterials will be limited by the lack of senior personnel with expertise in cell biology. This limitation is in part due to the difficulty in redefining the mission or scope of an institute after it has been established.

Several ongoing projects deal with the theme of bioelectronics. Individual projects have addressed the integration of supported bilayers, polymer-supported bilayers, giant vesicles, cardiac myocytes, and neuronal cells with field effect transistors. These efforts are each characterized by a very sophisticated integration of materials science, electronics, physics, and chemistry. The group led by Dr. Offenhauser has done a superb job of initiating work with mammalian cells. The inclusion of experienced and senior cell biologists would make this group an undisputed leader in the area.

SUMMARIES OF TECHNICAL PROJECTS

The scientists at this institute have developed a chemical approach to modify surfaces and electronics with lipid bilayers. Current efforts are aimed at integrating membrane proteins with electrical elements to realize sensors.

They have an excellent program in integrating neuronal cells with microelectrode arrays. Their work has largely solved the problem of patterning neurons and synapses, although there is still some work remaining to better control where synapses form between adjacent neuronal cells. They have focused on creating arrays of transistors for exciting and monitoring activities in cells. They have developed the electronics and data collection/analysis protocols for exciting and recording electrical activities in neuronal cells. The goals of these technical advances are to pattern many neurons into simple neural networks and study the training of these nets to perform simple computations. Related work with brain slices is already characterizing simple computations.

Site: **MeVis**
University of Bremen
Universitatsallee 29
28359 Bremen, Germany
http://www.mevis.de
http://www.mevis.uni-bremen.de

Date: 19 July 2000

WTEC Attendees: W.R. Wagner (report author), S. Gould, F.G. Heineken, J. West

Hosts: Dr. Guido Prause
Tel: 0421-218-28 76; Email: Prause@mevis.de

BACKGROUND

Founded in 1995, MeVis is the Center for Medical Diagnostic Systems and Visualization at the University of Bremen. It is a nonprofit organization of 28 employees, who primarily have backgrounds in computational science, physics, math, and engineering. MeVis is focused on developing software for improved clinical analysis of medical images, specifically in the evaluation of breast, liver, lung, brain, and vascular tissue. There is also an effort to develop educational and training software in the area of clinical image analysis. A for-profit company, MeVis Technology, was founded in 1997 to commercialize software and other technology developed at MeVis. Technology and development efforts transferred to MeVis Technology are sold, and MeVis Technology can contract for further development work with MeVis.

MeVis is not currently involved in what would be considered tissue-engineering-related research, although its technologies and interests may ultimately be applicable to the evaluation of tissue-engineered constructs *in vivo*. The company has developed software for the analysis, visualization, and manipulation of the large data sets associated with temporal, 3-dimensional images. A key asset has been the development of wavelet-based image compression methodologies. Specific projects include 3D temporal image analysis of contrast perfusion through breast tissue, which can allow the detection of tumors by evaluation of regional contrast filling rates or by quantitative analysis of other image data. Similar methodologies can detect regions of infarction in brain tissue. Another major effort is in the volumetric segmentation of liver and lung tissue based on dependency of filling by detected vascular trees. This analysis is useful in the surgical planning of tumor resection procedures and liver splitting for hepatic transplantation. One could envision that such analysis might be applied in the evaluation and modeling of tissue construct perfusion following implantation or the optimization of tissue construct placement to interface with existing vascularization.

R&D ACTIVITIES BY TOPIC

Engineering Design

The techniques for tissue/vascular segmentation might provide essential data for the development of mass transfer models. The ability to temporally track 3D structures *in vivo* would similarly benefit biomechanical modeling.

Informatics

Imaging Tools. MeVis is focused on data analysis, visualization, and manipulation, and works with medical images collected by currently utilized techniques.

Remote Interactions. The data compression methods utilized by MeVis make remote interactions more feasible. Remote image manipulation and expert evaluation using internet/high-bandwidth internet/wireless may be a future direction for the company. Its scientists are interested in the formation of "networks of competence."

Image Data Analysis. Through MeVis Technology, several commercial products have been produced.

Genomics/Proteomics and *Computational Biology/Chemistry*. MeVis has been working on three projects that might fall into one or more of these categories: (a) signal correction in magnetic resonance spectroscopy; (b) transcutaneous measurement of the hematocrit using laser light; and (c) automated analysis of 2D gel electrophoresis images.

LEGAL/REGULATORY ISSUES

European legal regulation and its conversion into German law were mentioned. This has had an impact on the imaging tools under development, in particular for soft-copy reading of mammograms. There did not appear to be a significant regulatory burden on the software under development.

GOVERNMENT ACTIVITIES

Funding from the state of Bremen has been essential to funding the nonprofit MeVis. This funding has been in place for the first 5 years of the organization and was to continue for another 4 years. The funding is tied to payments received by Bremen from economically stronger German states. Industrial funding has picked up and now is a major part of MeVis' budget.

REFERENCES

University of Bremen. About MATEC. http://www.matec.uni-bremen.de/institute/institute.html. 07/19/00. Handout.

_____ Research fields at MATEC. http://www.matec.uni-bremen.de/research/research html. Handout. 07/19/00.

_____ Center for environmental research and technology an interdisciplinary center presents itself. Brochure.

Site: **Smith & Nephew Group Research Centre**
York Science Park
Heslington, York YO10 5DF
UK

Date: 19 July 2000

WTEC Attendee: David Smith (report author)

Hosts: Dr. John Lang, Corporate Product Safety Assurance Manager
Alison Dale, Regulatory Affairs Manager, Wound Management
Michael Cox, Senior Medical Device Specialist, Medical Device Agency, UK

BACKGROUND

Smith & Nephew is a publicly traded manufacturer and distributor of medical products (primarily for orthopaedic applications) worldwide. It supports the development of various human tissue products through its partnership with Advanced Tissue Sciences, Inc.

This author attended the Tissue Engineering Regulatory Affairs Seminar organized by Smith & Nephew in conjunction with its annual Tissue Engineering Symposium and attended and participated in presentations regarding approaches to regulation of medical products incorporating human tissues within EU and UK (presentations given by Alison Dale, John Lang, and Michael Cox).

DISCUSSION

See *EU Regulation of Medical Devices and Medicinal Products* for a more comprehensive consideration of the treatment of engineered tissue products within the EU and among its member states.

Presentations given during this seminar provided a comprehensive overview of the inconsistencies in the classification of medical products incorporating human tissues among the member states in the absence of an EU classification decision. National classification schemes, to the extent such products are recognized and regulated at all, depend upon the source of the tissue (autologous vs. allogeneic) and its viability upon implantation. National reimbursement plans generally favor "medicinal products" over "medical devices"; least favored are unregulated products.

The FDA's Proposed Approach to the Regulation of Cellular and Tissue-Based Products was reviewed as an example of a comprehensive classification and scaled regulatory impact scheme. To the extent the Proposed Approach provides for the classification of engineered tissue products as either medical devices or "medicinal products" ("biologics" or "drugs" under U.S. medical products laws), its relevance as a model for EU regulation is uncertain. Nevertheless, as a means of identifying and implementing a uniform classification and marketing authorization process, the FDA's Proposed Approach does highlight the need for an EU legislative framework for tissue and cell-based products distinct from medicinal product or medical device regulations. While it can be anticipated that some engineered tissue products will be classified as medicinal products, most may remain unregulated within the EU for some time, despite the progress being made in other regions to develop new regulatory regimes recognizing the particular issues raised by and the attributes of these products.

Referring to the language of the EU Medical Devices Directive (Article 1, §5), Mr. Cox advised that the directive specifically excludes from its scope:

> (f) transplants or tissues or cells of human origin [and] products incorporating or derived from tissues or cells of human origin [and]
>
> (g) transplants or tissues or cells of animal origin, unless a device is manufactured utilizing animal tissue which is rendered non-viable or non-viable products derived from animal tissue.

By way of illustration, Mr. Cox compared products incorporating biomaterials intended to promote ingrowth of cells or cellular materials following application (e.g., a dermal regeneration template of collagen and GAG matrix for fibroblast infiltration and a hyaluronate-based system with biodegradable matrix), which are regulated as medical devices, with products delivering cells or tissue substances (e.g., a tissue-engineered matrix with keratinocytes and fibroblasts in a two-layer scaffold, and a bioabsorbable scaffold with growth factors and matrix proteins from human fibroblasts), which, despite the substantial identity of therapeutic indication, are not regulatable as medical devices. Consequently, in the absence of some formal process to classify an engineered tissue product as a "medicinal product," the only tissue products presently regulated under EU Directives are those incorporating or derived from nonviable animal tissues.

Mr. Cox emphasized that the differentiation between a "medical device" and a "medicinal product" may turn upon several factors: product description; intended use; manner of presentation; primary mechanism of action; scientific data on action; labeling information; and sponsor claims and statements about the product in presentations or promotional literature. Mr. Cox observed that opinions on the most appropriate regulatory route for innovative products may differ significantly; that the existing European regulatory framework does not yet encompass all types of medical products utilizing material of human or animal origin; and that all interested persons should remain alert to rapid developments through various initiatives underway in this area. (He noted, in regard to this last, the establishment of a Tissues Working Group within the UK Medical Devices Agency and the pendency of UK tissue banking regulations). Nevertheless, he anticipates litigation involving the classification of medical products derived through tissue engineering.

In anticipation of or to encourage the development of new, uniform regulatory approaches for engineered tissue products, substantial consideration was given during the seminar to the development of product standards under the auspices of standards organizations in the United States and Europe. The comprehensive scope of the ASTM TEMPS (tissue-engineered medical products) standards development program was reviewed, with encouragement that European companies and regulatory bodies should participate in this program as an international vehicle and forum for coordination of scientific and technical criteria for marketing authorization. In his comments, though, Mr. Cox noted that the definitions of tissue engineering an engineered tissue product can have a significant impact on the applicability of current regulatory paradigms.

Site: **University of Freiburg**
Chirurgische Universitatsklinik
Hugstetter Strasse 55
D-79106 Freiburg, Germany

Date: 21 July 2000

WTEC attendees: L.V. McIntire (report author), H. Griesler, D. Mooney

Hosts: Prof. Dr. med. G. Björn Stark
Tel: 0049-761-270-2817; Fax: 0049-761-270-2501
Email: STARK@ch11.ukl.uni-freiburg.de

BACKGROUND

The Valley Tissue Engineering Center (ValleyTEC) is part of the BioValley project, which is designed to promote the infrastructure of the tri-national (France, Germany, Switzerland) upper Rhine Valley. ValleyTEC unites regional businesses, entrepreneurs, and investors with researchers at the University of Freiburg, headed by Prof. G.B. Stark of the Department of Plastic and Hand Surgery. Other departments at the University Medical Center Freiburg are also involved, including the Department of Orthopaedics and the Department of Dermatology. Prof. Stark is a plastic surgeon who had begun developing tissue-engineering approaches to skin repair in 1990 and extended his research to cartilage and bone and gene therapy approaches in 1994-95. The group organized the Congress on Tissue Engineering that attracted 300 participants in 1997 and had approximately 600 people attending in 1999. In 1998 the local state government gave initial three-year funding to establish ValleyTEC. The initial grant was DM 5.4 million over three years, with about 50% state and 50% private sector funds. This does not include other external funding, which is currently in the range of approximately DM 2 million. Major contributors include Baxter.

The largest group of investigators in ValleyTEC are surgeons, who use established biomaterials, primarily collagen and fibrin gels, as the scaffolds for cell incorporation. Dr. Stark has been utilizing keratinocytes in fibrin glue since 1991. Bovine and equine collagens have also been used. Overall the group is not strong in materials science, although there are plans to add new faculty members in applied science at Freiburg, who may strengthen this area.

There is an active Center for Technology Transfer at the University of Freiburg. One company has already been established through ValleyTEC-Biotissue Technologies (BTT). The center has a GMP facility for expansion of autologous cells. The major product is expanded numbers of keratinocytes from patients, and BTT works closely with plastic surgeons in Dr. Stark's group. It should be noted that the city of Freiburg has built the "Biotech Park-Freiburg" for helping spin off biotechnology companies; it can provide quality space, marketing, sales advice, and other services.

The tissue-engineering research group with the Department of Plastic and Hand Surgery consists of 25 medical students, 5-6 residents, 3-4 international fellows, and 3-4 technicians. There is a strong desire to get results to the clinic. The keratinocyte/fibrin glue materials were used in human applications before being tested in mice or other animal models. In Germany, medical doctors have strong freedom of clinical practice. Clinical protocols are approved by university ethical committees or regional medical boards. Litigation is not prevalent in medical practice in Germany.

R&D ACTIVITIES

Dr. Stark's group also has interests in a wide range of potential tissue-engineered products, including

- Co-culturatins of endothelial cells and keratinocytes
- Osteoblasts on collagen gels

- Endothelial cell-lined collagen tubes for small vessel graft replacement
- Neo-urethra
- Peripheral nerve regeneration
- Striated muscle
- Gene therapy approaches
- Drug delivery applications (collagen with plasmids, fibrin with EGF)

Dr. Stark feels that regional centers will eventually be established to provide expanded autologous cells for a wide range of tissue-engineered applications. Currently, embryonic stem cell research is quite restricted in Germany, and human applications of gene therapy are difficult to get approved.

Biomaterials

The center uses collagens and fiber glues; this is not an area of strength.

Biomolecules

Center scientists believe the "body is the best reactor they have" (Vacanti approach). There is no special strength here.

Cells

Use of aAutologous cell expansions are used principally. There is ongoing clinical work in keratinocyte/fibrin glue constructs, and cartilage or bone (collagen scaffold) work was anticipated. Other projects are less developed currently.

Bioengineering

Biomechanics is not a strength, although they do measure mechanical properties of their cartilage construct. The company GMP facility, Biotissue Technologies, is used for cell expression.

REGULATORY ISSUES

There is involvement of both federal and state government in developing and funding ValleyTEC, with strong private company involvement. The center's concept of taking products directly to the clinic is interesting; there is strong control by medical doctors and little fear of litigation.

Site: **University of Koln**
Institut für Neurophysiology
Robert Koch Strasse 39
D-50931 Koln, Germany

Date: July 20, 2000

WTEC Attendees: Milan Mrksich (report author)

Host: Dr. Bernd Fleischmann (Prof. Heschler was away from the Institute).

SUMMARY

Rresearchers at the Institute for Neurophysiology at the University of Koln are mostly concerned with the fundamental issues of development cardiac and neuronal cells from embryonic stem cells. Their expertise is in characterizing the electrophysiology of the developing cells and in applying excellent cell biology to the problem. Notably, they have brought the techniques of immunology to studies of development in electrically active cells. The institute has less expertise in engineering and materials, though these areas are secondary to the primary research programs. The program has a clear connection to transplantation, but the emphasis is mainly on understanding the biology of development and emergence of electrical activity in cells and tissue.

R&D ACTIVITIES

The WTEC team met with several researchers and received briefs on several projects. These meetings are summarized below.

Dr. Kathrin Banach cultures ES-derived cardiac cells on microelectrode arrays in order to study the emergence of cell beating. This project uses commercial arrays through a collaboration with Multichannel Systems. The team can grow the embryoid bodies on the arrays and record signals from multiple channels. This work is providing first-rate advances in understanding heart development. Overall, the effort is very strong in the biology, but less sophisticated in issues of electronics and surface chemistry.

Dr. Toni Schneider described the characterization of a new voltage gated calcium channel. The channel protein is mainly expressed in neuronal tissue and is believed to be involved in insulin production, but it has no phenotype (from knock-out experiments). This team is studying the electrical properties of the channel with the aim of understanding the electrophysiology in cells and tissue.

Dr. Fleischmann hosted a lunch where the discussion centered on the broad issue of multidisciplinary research in Germany. There is a general difficulty in promoting collaborative efforts across different fields (biology and engineering/physics), and there has been little interaction across these fields to date. Similarly, while industrial interactions with academic laboratories are encouraged, these have been rare in the biological sciences. Indeed, the multidisciplinary element of research is one of the main differences between U.S. and European science, with the advantage going to the United States.

Dr. Nibedita Lenka presented a general overview of the differentiation of ES cells into neurons. The use of tissue-specific promoters is an important tool for investigating differentiation.

Dr. Susanne Ullrich is studying the neural regulation of insulin secretion in beta cells. The exocytosis of insulin from vesicles is triggered by the closure of a potassium channel and a resulting cell depolarization. Stem cells are engineered with an insulin-specific promoter to express GFP to visualize development of these cells in embyroid bodies.

Dr. Heinrich Sauer discussed new methods for culturing the ES cells. This team has developed methods based on the spinner flasks to avoid the low throughput that is common with hanging drop cultures. Dr. Sauer is also interested in getting higher yields of cardiac cells (10-20% is current best; quantitative

desired). His group is also working on the challenging problem of devising methods for purifying differentiated cells at the complete exclusion of non-differentiated cells, since the latter can lead to tumor formation in sites of transplantation. Dr. Sauer also discussed recent work that uses a proximal electrode to stimulate the differentiation of cardiac cells. The observation that electrical field (or the electrogeneration of specific molecules) can affect cell differentiation is exciting but still awaits a mechanistic explanation.

Overall, this institute excels in a fundamental cell biological approach to understand the development of electrical activities in embryoid bodies. The applied studies that address tissue engineering or medicine are secondary but may find a larger role in the future programs. The institute has an excellent integration of biologists and biophysical researchers (primarily electrophysiologists) but does not link strongly to engineering or physical sciences (of course, that is not their mission). While the institute has an interest in interacting more closely with researchers in these areas, the culture and infrastructure make it difficult.

Dr. Fleischmann was most kind and cooperative in hosting our visit.

Site: **University of Regensburg**
Institute of Pharmaceutical Technology
Dept. of Chemistry
93040 Regensburg, Germany

Date: November 2000

WTEC Attendees: D. Mooney (report author)

Host: Dr. Achim Goepferich

INTRODUCTION

The WTEC visit to Dr. Goepferich's laboratories involved informal discussions of the current state and future of tissue engineering in Germany, and one-to-one meetings with the investigators in his group. Dr. Goepferich's laboratory has a strong focus on tissue engineering, with emphasis on biomaterials aspects of this field. He established the labs three years earlier, and the lab consisted of 23 members at the time of the WTEC visit. Dr. Goepferich agreed with the previous findings of the panel that the emphasis in Germany is autologous cell therapies. He believes Germany is at an early stage of tissue-engineering research, as compared to the United States, and he feels progress in Germany is impeded by very limited funding focused on tissue-engineering research.

SCIENCE

Biomaterials

The focus of research in these laboratories is the development of PEG-PLA polymers as delivery vehicles for inductive protein and cell delivery. There are significant efforts to control protein and cell interaction with these polymers and thus to regulate tissue development by variation in the polymer composition. This laboratory has demonstrated that bone development may be regulated by the polymer composition, and it is developing novel methods to fabricate three-dimensional polymer scaffolds from these polymers and utilize these scaffolds in the transplantation of several cell types. New polymers, based on the same backbone chemistry, are also being developed in which cell adhesion molecules or peptides may be covalently bound.

Biomolecules

Dr. Goepferich's group is doing work in several areas of biomolecules research. Researchers in this group are studying the role of several proteins, including hedgehog and insulin, on the development of engineered cartilage. In these studies the proteins are added to the culture medium. They are also developing PEG-PGA polymers to which they can covalently couple growth factors. The premise underlying their work is that presentation of immobilized growth factors may allow them to tightly regulate gene expression of cells interacting with the polymers. Finally, this group is developing microsphere and nanoparticle delivery vehicles for proteins. These systems may find great utility in tissue engineering and general drug delivery applications.

Cells

A variety of cell types are being used to engineer several tissue types. This group is utilizing mesenchymal stem cells to engineer both bone and adipose tissue. It is attempting to control the fate of the cells by both growth factor presentation and the chemistry of the scaffolds. It is also utilizing chondrocytes to engineer cartilage.

Engineering Design

Transport issues

This group is very interested in the issue of promoting vascularization in engineered tissues. One approach is the delivery of angiogenic molecules utilizing their polymeric delivery vehicles. It is beginning to study endothelial cell transplantation as a means to promote capillary formation in engineered tissues.

Biomechanics

This laboratory has adapted elements of the bioreactor technology developed in the Langer lab (MIT, U.S.) to grow three-dimensional tissue constructs.

Site: **University of Twente**
Postbus 217
7500 AE Enschede
The Netherlands

Date: 21 July 2000

WTEC Attendees: J. West (Report author), S. Gould, F.G. Heineken, W. Wagner

Host: Dr. D.W. Grijpma, Dept. of Chemische Technologie
Tel: +31 53 489 2966, Email: d.w.grijpma@ct.utwente.nl

SUMMARY

We did not visit this site but met with Dr. Grijpma at IsoTis. He is a professor in the department of chemical technology in the Biomaterials Research Center at the University of Twente. This center brings faculty from many disciplines together to pursue research in cell-material interactions, development of new polymers, endothelialization of vascular grafts, and surface modification of biomaterials. Dr. Grijpma collaborates with IsoTis and indicated that half of all research in science/engineering is industrially funded in Dutch universities.

APPENDIX C. SITE REPORTS—JAPAN

Site: **Hokkaido University**
School of Medicine
Nishi 7, Kita 15-jo, Kita-ku
Sapporo, 060-8638, Japan

Date Visited 25 August 2000

WTEC Attendees: C.A. Kelley (Report author) , D.J. Mooney, H. Morishita, A.J. Russell, D. Smith

Hosts:
Satoru Todo, MD, Professor and Chairman, The First Department of Surgery, Hokkaido University School of Medicine
Michiaki Matsushita, MD, PhD, Associate Professor, Department of Surgical Therapy, Hokkaido University, Postgraduate School of Medicine
Yoshihito Osada, PhD, Professor, Division of Biological Sciences, Graduate School of Science, Hokkaido University
Dr. Masatsugu Shimomura, Laboratory Head, Dissipative-Hierarchy Structures Laboratory, Spatio-Temporal Function Materials Research Group, Frontier Research System, RIKEN

INTRODUCTION

The group the WTEC team met with consisted of representatives from several different departments within Hokkaido University and one representative from RIKEN. Dr. Todo, a transplant surgeon, assembled this multidisciplinary group based on his experience working in the United States, where he learned the value and need for multidisciplinary teams for certain areas of biomedical research. He indicated that in Japan, medical doctors do not typically work with basic scientists. Dr. Todo led the informal discussion among those present. In addition to himself, a transplant surgeon, his team consists of Dr. Matsushita, who is interested in developing artificial liver support systems; Dr. Osada, whose interest is in robotics and artificial muscle systems made with ultra water-absorptive polymer gels; and Dr. Shimomura, who is working on micropatterning of polymer substrates for use as tissue-culturing scaffolds. The group Dr. Todo assembled submitted a tissue-engineering proposal to one of the government ministries, but it was not funded, mainly because the group was proposing to work on too many organ systems. He explained that research in tissue engineering at Hokkaido University is presently in an infantile stage.

Dr. Todo talked mainly about his current research and clinical practice, which is related to organ transplantation. Most organ transplants in Japan over the past 20 years have been from living donors. Three years ago, the first cadaver transplant from a brain-dead donor was allowed, which was a liver transplant. Additional cadaver transplants have been performed over the past 3 years, but by far the majority of organ transplants are still from living donors. Dr. Todo's research is focused on the effects of novel immunosuppressants on organ transplantation, organ preservation for transplantation, and methods for reducing liver damage caused by ischemia and reperfusion. In the future, the results of this work should be of relevance to engineered tissues and organs.

Dr. Matsushita spoke of extracorporeal liver support systems. The survival rate for acute liver failure used to be approximately 20%. Since the use of extracorporeal purification systems as a bridge to transplant, the survival rate has increase to 60-70%. He believes that future directions should include the development of tissue-engineered purification systems, and he indicated that this is being pursued by researchers at Hokkaido University as well as at Kyoto University and the University of Tsukuba. He thought that making transgenic pigs to generate livers was too expensive and the success rate too low.

R&D ACTIVITIES

Biomaterials

The group, particularly Dr. Shimomura, is interested in developing a scaffold for tissue-engineered livers. He is making honeycomb, micropatterned polymer films immobilized onto glass plates. The micropatterning is on the 10 micron scale. The substrate is very flexible, and thus he is able to make any 3-dimensional structure. He is currently using this film to make scaffolds for tissue engineering. The polymer he is using is not new, but the method he is using to cast the polymer film is novel, and thus he can create new properties using existing materials.

According to Dr. Osada, there was limited discussion among the group on artificial joints and tissue-engineered joints. Dr. Osada pointed out that artificial joints work well over a limited time frame of about 10-15 years, but this is too short and revisions do not work well. He and his colleagues felt tissue-engineered joints were a long way off. They mentioned that they have developed new methods to make hydrogels with the necessary friction coefficients for the type of load and wear imposed by joints. They also said that they have some ideas about how to chemically attach these hydrogels to bone to overcome the problem of integration of engineered tissue with host tissue under long-term loading.

Cells

No work in this field was described, but there was a brief discussion of islet cells. Our hosts said that human sources of islet cells are very limited. They were concerned about diseases from xenotransplantation. They felt that growing islets will eventually be possible.

Biomolecules

Our hosts indicated that they were doing research in this area but they did not discuss what they were doing.

Engineering Design Aspects

Our hosts did say that they were working on the problem of mass transport in 3-dimensional tissues constructed of cells in hydrogels, but they did not go into any further detail on research they were doing in this area.

Bioinformatics

Dr. Osada briefly mentioned his studies involving the use of robotics with catheters. He is doing animal experiments using this technology and explained that in humans he envisions its usefulness in NASA applications and other situations where human beings cannot do the manipulation. This work has been supported by the Japanese government for the last 15 years, mainly by MITI and the Ministry of Education. Beyond this work, our hosts indicated that they have no plans to develop imaging or informatics technologies. They will use existing technologies.

Site: **Japan Tissue Engineering Company, Ltd**.
6-209-1 Miyakita-dori
Gamagoori, Aichi 443-0022, Japan
http://www.jpte.co.jp

Date visited: 23 August 2000 [this report includes revised data provided by the host in September 2001]

WTEC Attendees: A. Russell (report author), C. Kelly, D. Mooney, H. Morishita, D. Smith

Hosts: Mr. Toshihiro Osuka
Tel: 81-533-66-2020; Fax: 81-533-66-2019; Email: tosk@jpte.co.jp
Dr. Kentaro Takamura
Tel: 81-533-66-2020; Fax: 81-533-66-2018; Email: kenta@jpte.co.jp

INTRODUCTION

Japan Tissue Engineering Company, Ltd. (J-TEC) was founded in February 1999 as an early-stage but committed worldwide tissue engineering company. J-TEC estimates a $7 billion market by 2020 for its products in Japan.

J-TEC is owned by NIDEK, a medical device manufacturing company, and is the only tissue engineering company in Japan. It was initiated by a government loan of $9 million from the Ministry of Health, Labor, and Welfare (formerly the Ministry of Health and Welfare), and capitalization of $4 million, which was increased to $7 million in 2001. NIDEK sells optics and lasers in more than 90 countries. Other founding partners are Toyama (a pharmaceutical company), INAX (a construction company), and Tokai Bank Group.

J-TEC has 7 PhD-level and 12 MS-level researchers. J-TEC has submitted 39 patents and occupies 16,000 square feet of state-of-the-art lab space, including animal research facilities, pathology, electron microscopy, biohazard, cell bank, and cultivation/inspection facilities.

J-TEC has a very aggressive proposed product pipeline, starting with sales of non-patented auto-keratinocyte sheets. Skin and cartilage will be the first products, then CNS, then all others.

J-TEC collaborates with many national projects, and in particular, with its founder, Dr. Ueda at the Department of Oral and Maxillofacial Surgery of Nagoya University.

R&D ACTIVITIES

Biomaterials

J-TEC is using existing approved materials and is not currently searching for novel matrices. For this reason, liability issues seem minimal. The biomechanics of matrices are of special interest, given that one of the founding companies is in the construction business.

Cells

Allogeneic cells are obtained from universities, and in the future, a government-founded non-profit is likely to coordinate the distribution of tissues.

Relatively little work is going on concerning scale-up issues for cell expansion, but J-TEC will focus on this later.

J-TEC has patent filing ready for novel tissue preservation techniques.

The main distinguishing feature of J-TEC is its novel approach to CNS stem cell isolation and expansion. Its scientists are working hard on vascularization issues and "three-dimensional tissue engineering."

REGULATORY ISSUES

J-TEC sees that the regulatory path will follow the lead of how Advanced Tissue Sciences' application for a clinical trial is handled by the Ministry of Health, Labor, and Welfare (MHLW). MHLW is likely to follow the lead of the FDA.

J-TEC will work with adult-derived stem cells rather than embryonic ones.

The keratinocyte sheet that was to come on the market shortly after the WTEC team's visit was to be regulated as a device, needing only a one-stage clinical trial.

References

Japan Tissue Engineering Company, Ltd**.** n.d. *J-Tec.* Brochure.

——. 1999. *Corporate Outline*. Brochure.

Toyama Chemical Co. n.d. *Toyama Chemical Co., Ltd. Company Profile*. Brochure.

NIDEK. n.d. *Nidek Company Profile*. Brochure.

Site: **Keio University**
Laboratory for Bioinformatics
5322 Endo
Fujisawa, 252-8520 Japan

Date visited 26 August 2000

WTEC attendees: D. Mooney (report author), C. Kelley, H. Morishita, A. Russell, D. Smith

Host: Masura Tomita, PhD, Professor and Director, Laboratory for Bioinformatics
Tel: +81- 466-47-5111, x53230; Fax: +81- 3-3440-7281;
Email: mt@sfc.keio.ac.jp

SUMMARY

Dr. Tomita is heading up a project to develop a computer model that can simulate all functions of a cell: the E-Cell project. His lab is based at Keio University, which has 8 campuses in the Tokyo area. This specific campus was constructed in 1990, and houses IT. A striking feature of the campus is a number of free-standing cottages, each of which houses a single research group. These cottages contain small wet labs, computer rooms, individual cubicles, kitchen and recreation room, bedroom, and conference room. These cottages are not intended to serve as living facilities, but provide for the needs of researchers during extended work hours. Dr. Tomita's research group is comprised of approximately 25 people and is mainly funded by the Ministry of Science and Technology.

The WTEC team's visit was quite short, less than 1 hour, and was mainly comprised of a presentation by Dr. Tomita on his cell simulations. A summary of this presentation is given below. This work may find relevance to tissue engineering in several areas. Extension of these models to cell populations may make it possible to determine the role of various external signals (e.g., growth factors, mechanical signals) and cell-cell interactions on tissue development and function. This could in turn make it possible to readily screen and develop new tissue regeneration and engineering strategies.

R&D ACTIVITIES

This work did not have direct relevance to areas of this WTEC panel other than in the area of informatics.

Informatics

The concept of the approach is that cell function is a collection of a large number of chemical reactions, but each reaction can modeled by simple reaction pathways leading to complex overall behavior. The first approach developed by Dr. Tomita used a virtual cell expressing 127 genes, with 4268 molecular species and 495 reactions. Sequencing of M-genitalium was first thought to provide a model organism, but the large number of unknown function genes created too many difficulties. Instead, a set of genes was selected that were believed to comprise a critical set of functions. Rate constants were taken from the literature, if available, or estimated if unknown. This model was designed to take into account enzymatic reactions, transport (e.g., glycerol into cell), stochastic behavior, and diffusion inside the cell. This model has now been used to determine the effects of various experimental conditions on cell metabolism. This model is available for downloading via the web (http://www.e-cell.org) and has been downloaded over 300 times. This group is now working on a number of new cell models capable of more complex behavior. These include self-sustaining models and a model of erythrocytes.

Site: **Kyoto University**
Institute for Frontier Medical Sciences
53 Kawahara-cho Shogoin, Sakyo-ku
Kyoto, 606-8507 Japan

Date visited: 24 August 2000

WTEC Attendees: C.A. Kelley (report author), D.J. Mooney, H. Morishita, A.J. Russell, D. Smith

Hosts: Professor Yuji Hiraki
Professor Hiroo Iwata, Tel: +81-75-751-4119; Fax: +81-75-751-4144;
Email: iwata@frontier.kyoto-u.ac.jp
Professor Masanori Oka
Professor Yasuhiko Shimizu
Professor Yasuhiko Tabata
Associate Professor Naohide Tomita
Professor Sadami Tsutsumi

INTRODUCTION

The Institute for Frontier Medical Sciences (IFMeS) at Kyoto University has its origins in its formal recognition as the Medical Polymer Study Group in 1978. In 1980 this Study Group became established as the Research Center for Medical Polymers; in 1990 the Center's name was changed to the Research Center for Biomedical Engineering; and in 1998, the Research Center became the Institute for Frontier Medical Sciences. IFMeS is an interdisciplinary institute encompassing five major research fields, each represented by four academic research departments:

1. Field of Biological Function, which includes the Departments of Molecular and Cellular Biology, Ultrastructural Research, Experimental Pathology, and Medical Simulation Engineering
2. Field of Tissue Engineering, which includes the Departments of Molecular Interaction and Tissue Engineering, Biomaterials, Reparative Materials, and Mechanical Properties
3. Field of Regeneration Control, which includes the Departments of Development and Differentiation, Medical Embryology and Neurobiology, Growth Regulation, and Immunology
4. Field of Medical Systems Engineering, which includes the Departments of Medical Systems Engineering, Biomechanical Engineering, Medical Systems Control, and Medical Engineering
5. Field of Clinical Applications, which includes the Departments of Biological Repair, Tissue Regeneration, Organ Reconstruction, Bioartificial Organs, and Regenerative Medicine.

In addition, the institute has the Laboratory of Animal Experiments for Regeneration.

R&D ACTIVITIES

Biomaterials

Dr. Iwata is addressing issues associated with the tissue/biomaterial interface. He has created self-assembled monolayers on gold-coated glass plates and perfused them with plasma. Using a laser beam, he looks at the angle of deflection of the refracted light to see how much protein is adsorbed on the surface. Using antibodies, he is beginning to identify the adsorbed proteins; thus far he has identified C3b adsorption.

The main focus of Dr. Tabata's research is on drug delivery systems for *in vivo* tissue engineering. He has been using biodegradable gelatin hydrogels for the controlled release of growth factors to the site of regeneration *in vivo* to stimulate cell proliferation and differentiation. The gelatin hydrogel is biologically safe, and its high level of inertness towards protein drugs prevents the denaturation of the protein, which is a

common problem with polymer protein delivery systems. Also, controlled release of proteins from non-biodegradable hydrogels over a long time period would not be expected because the rate of protein release is generally diffusion-controlled through aqueous channels within the hydrogels.

Thus, Dr. Tabata's approach of using a biodegradable hydrogel to immobilize growth factors allows for the release of the factor through hydrogel biodegradation. The rate of degradation is controlled by changing the extent of cross-linking, which produces hydrogels with different water contents. The higher the water content, the faster the *in vivo* degradation and rate of release of protein. In addition, instead of using chemical methods to immobilize growth factors to the delivery system, which often results in protein denaturation, he uses physical coupling based on charge. The bioabsorbable gelatin hydrogel can be made either with a negative or a positive charge based on the processing method, and the growth factor is electrostatically complexed with the polymer chain to allow for physical immobilization in the hydrogel carrier. In one study, gelatin hydrogels incorporating bFGF were subcutaneously implanted into the backs of mice, which resulted in neo-vascularization. The potential applications of this technology that Dr. Tabata is considering including vascularization to ischemic tissue and to transplanted cells or tissues for organ substitution. He has also designed gelatin hydrogel microspheres coupled to bFGF for injection. When injected into the infarction site of dog hearts, collateral coronary arteries formed. His plan is to use the biomaterial designs with a number of growth factors and for a number of applications. He had some data showing that bFGF-incorporating gelatin hydrogels stimulate bone regeneration in a rabbit and monkey model of a skull defect. Controlled release of bFGF from gelatin microspheres was also effective in forming adipose tissue in the backs of mice.

Dr. Shimuzu's group is working on autologous tissue regeneration *in vivo* by providing scaffolds that promote cell proliferation and differentiation. The scaffolds being developed consist of extracellular matrix obtained by complete removal of cell components from allogeneic or heterogeneic organs or tissue. The de-cellularized matrix is mixed with reconstituted collagen types I, III, and IV, extracted from swine skin by enzyme treatment in a neutral solution to abolish immunogenicity. For reinforcement, the extracellular matrix is combined with synthetic biodegradable polymers. In some cases, cells and/or growth factors are added. Target tissues and organs include

- membranes, such as the cornea, pericardium, pleura, peritoneum, and dura matter of the brain
- tubular organs, such as the blood vessels, trachea, and digestive tubes
- tissues receiving external force, such as teeth, periodontal membrane, cartilage, bone, tendons, and ligaments
- neurological systems, such as the peripheral nerves and spinal cord
- urological systems, such as the bladder and ureters
- parenchymal organs, such as the lungs, heart, liver, and kidneys

Dr. Shimuzu presented the results of several studies using autologous tissue regeneration. For the pericardium, pleura, and dura mater of the brain, membrane sandwiches made of collagen and PGA, and coated with gelatin, were used in dogs to regenerate defects in endogenous membranes. After 2 months, tissue regeneration was observed. A new prosthesis was designed for reconstruction of the bifurcation of the trachea. The prosthesis consists of a Y-shaped Marlex mesh tube reinforced with polypropylene spirals and coated with collagen. He has had limited success in dog experiments, but regeneration of the tracheal epithelium has been observed to some degree, and additional improvements of the prosthesis are in progress.

An artificial esophagus is also under development. It is composed of a collagen sponge tube into which a silicone tube has been inserted. A 5 or 10 cm length of the esophagus was replaced with the prosthesis using end-to-end anastomosis in dogs. After implantation, the dogs were fed intravenously for 4 weeks. The silicone tubes were then removed and oral feeding started. After 1 month, much of the structure of the esophagus was restored, including the circumferential and longitudinal muscle layers.

Nerve regeneration is also being studied. Dr. Shimuzu's group examined nerve regeneration across a long gap in the dog peroneal nerve using a novel artificial nerve conduit. The conduit consists of a PGA collagen tube filled with laminin-coated collagen fibers. The nerve conduit was implanted across an 80 mm gap in the

peroneal nerve. After 3 months the dogs could walk with a limp, and after 6 months they walked normally. Microscopic observations at 12 months showed numerous myelinated nerve fibers, although the fibers were smaller in diameter and had a thinner myelin sheath than normal nerve fibers. These results show the potential usefulness of this artificial nerve conduit in enhancing peripheral nerve regeneration even across large gaps.

Dr. Oda's laboratory is involved in the development of artificial articular cartilage for articular resurfacing and joint replacement. Scientists in his group developed a poly (vinyl alcohol) hydrogel (PVA-H) which is a rubber-like gel, and recently, they improved the mechanical properties of the gel through a new synthetic process. They found that the gel has good biomechanical and biocompatibility properties for use as artificial articular cartilage. PVA-H had a thicker fluid film under high pressure than did polyethylene (PE). PVA-H also had a lower peak stress and a longer duration of sustained stress than PE, suggesting a better damping effect. The wear factor of PVA-H was approximately five times that of PE. Histological studies in animals showed no inflammation or degenerative changes in the PVA-H after 52 weeks of implantation. They found that PVA-H does not attach to the bone in the joint; however, the artificial articular cartilage made from PVA-H could be attached to the underlying bone using a composite osteochondral device made from titanium fiber mesh. Implants made of PVA-H on a titanium fiber mesh were used to replace the femoral condyles of dogs. The damage to the tibial articular surface was then studied. The hydrogel composite caused minimal damage to the articular cartilage and menisci. The composite osteochondral device became rapidly attached to host bone by ingrowth into the supporting mesh. Dr. Oda's laboratory is also investigating the possibility of making artificial intervertebral disks using PVA-H. He thinks this material will be useful for replacing one side of a joint but not both sides.

Cells

Dr. Hiraki's research is focused on understanding the molecular mechanisms regulating cartilage differentiation during endochondral bone formation. He is exploring this using both an *in vitro* and an *in vivo* model of chondrogenesis. His *in vitro* model uses the mouse embryonal carcinoma-derived clonal cell line ATDC5, because isolated chondrocytes do not maintain a differentiated phenotype beyond primary culture. ATDC5 cells possess the stem cell characteristics of self-renewal and high proliferative capacity exhibited by pre-chondrogenic mesenchymal cells and undergo the multistep differentiation process of chondrocytes during endochondral bone formation. This process spans from mesenchymal condensation to calcification. Dr. Hiraki is defining conditions for stimulating mesenchymal cell to chondrocyte differentiation. The various stages of chondrogenesis in the *in vitro* model include

1. pre-chondrogenic, the cells proliferate and then form a contact-inhibited monolayer
2. IGF1 or insulin is added to the cell cultures and the cells undergo condensation before overt chondrogenesis
3. cell proliferation decreases and cartilagenous nodules form; cells within the nodules become hypertrophic and express type X collagen; there is a dramatic increase in alkaline phosphatase activity; and eventually the cells undergo mineralization

The high frequency of conversion of cells to chondrocytes enables Dr. Hiraki to study the differentiation stages of the cells at the molecular level. Taking advantage of inductive chondrogenesis *in vitro*, Dr. Hiraki has found that during the growing, maturation, hypertrophy and calcification stages these cells differentially express various growth factors such as FGF, PTH, BMP-4, TGFbeta2, and BMP6. After the nodule stage, BMP-2/4 stimulates chondrocyte formation. The PTH/PTH-related peptide receptor is expressed during the early stages of chondrogenesis in parallel with the formation of cartilagenous nodules in culture, and is undetectable in undifferentiated cells. Addition of exogenous PTH or PTHrP at the contact inhibited monolayer stage, inhibited subsequent cellular condensation and formation of cartilagenous nodules. If PTH is added to the cultures at the nodule stage, the cells differentiate. These results suggest that activation of PTH/PTHrP receptors interferes with the early stages of chondrogenesis.

Dr. Tomita discussed his work related to regeneration of cartilage using either collagen gel containing chondrocytes or bone-marrow-derived mesenchymal cells. Transplanted chondrocytes embedded in collagen gels and cultured for 2 weeks were transferred to defective joints. Some regeneration of the joints was seen;

however, normal structure was not regenerated. Bone-marrow-derived mesenchymal cells in collagen gels did not work as well as chondrocytes.

Biomolecules

In addition to Dr. Hiraki's *in vitro* model of chondrogenesis, he is studying cartilage repair in rabbit knees in which full-thickness articular cartilage defects have been created. Articular cartilage has a limited capacity for regeneration and repair that is largely dependent on the size of the defect. If the defect is less than 3 mm in diameter, there is spontaneous regeneration of articular cartilage through migration and differentiation of chondroprogenitor cells from the bone marrow. If a neutralizing antibody to FGF2 is added to the defect in the rabbit knee, it results in fibrous tissue formation only. So his theory is that in defects 3 mm or less in diameter, FGF2 signaling plays a key role in the recruitment of chondroprogenitor cells and the maintenance of their high proliferative activity to support a chondrogenic repair response in the defects. In defects are greater than 5 mm in diameter, chondrogenic differentiation does not occur because of poor recruitment of osteochondral progenitor cells. Instead the defects are replaced by fibrotic tissue. If chondrocytes are added to full thickness defects in the knee joint, there is no coupling of the cartilage to bone. Dr. Hiraki is interested in promoting a repair response by supplementation of the signaling molecules that support proliferation of mesenchymal stem cells in large defects. In his studies he looked at whether FGF-2 can induce regeneration of full-thickness defects of articular cartilage. He found that administration of 50 pg of FGF2 for 2 weeks resulted in increased articular cartilage formation at 2-4 weeks and there was coupling to bone. He believes the mechanism involves recruitment of mesenchymal stem cells from bone marrow.

Engineering Design Aspects

One of Dr. Iwata's projects is aimed at developing a bioartificial liver as a temporary liver assist device. His design is a cartridge composed of 200 micron diameter hollow fibers inoculated with 100 grams of hepatocytes. The weight of a human liver is approximately 1000 g, but Dr. Iwata believes that only one-fifth of liver tissue is needed to replace liver function. One unique feature of his device is that it can be perfused with whole blood, whereas most artificial liver devices require plasma separation. The system is set up so that the blood coming from the pig flows through the bioartificial liver, outside of the hollow fibers which are closed off on both ends, and then through an oxygenator and back into the animal. Within the hollow fibers, the hepatocytes form rod-shaped cell aggregates during *in vitro* perfusion, and a bile canaliculus-like structure was occasionally seen between hepatocytes. High magnification showed that the canaliculus was separated from the remainder of the intercellular space by a tight junction. Together these observations suggest that the hepatocytes form functionally associated cell aggregates with a compact morphology not unlike hepatocyte spheroids. The bioartificial liver maintained the ability to metabolize lidocaine, ammonia, and galactose for 7 days in *in vitro* circulation and then deteriorated with time. The artificial liver functions for approximately 50 days. Dr. Iwata is now gearing up to begin human studies.

Dr. Iwata is also working on developing a bioartificial pancreas that is designed to incorporate islet tissue within agarose microcapsules (500 micron diameter) to prevent allo-and xenograft rejection. He reported on a study in which islets from hamsters were incorporated into agarose microcapsules and then transplanted intraperitoneally into *nod* mice, a diabetic mouse model. *In vivo* function was determined by glucose challenge. Before implantation, glucose levels remained high; after implantation, glucose levels decreased and were maintained for 60 days. One in five failed at 60 days and the others functioned out to 120 days. Failure was believed to be attributed to either immune rejection or fibrous encapsulation. Although this bioartificial device worked well in mice, it was not as successful in dogs. In addition, if the microbeads were xenogenically implanted in the subcutaneous tissue of the mice, the islets quickly died due to the lack of a vascular supply. To overcome this problem, Dr. Iwata designed experiments in which capillary bed formation was induced by controlled release of growth factors VEGF or FGF from hydrogels under the skin, and then the islet tissue was transplanted. In control mice, glucose levels remained high, whereas glucose levels were well maintained in mice with prior capillary induction.

Dr. Iwata also presented data on growing 3-dimensional tissues. He pointed out that one big limitation of growing 3-dimensional tissues is the diffusion of nutrients and oxygen into the tissue and the elimination of waste products. To begin to address this problem, he took hollow fibers coated with fibronectin and added to them bovine carotid artery endothelial cells and vascular smooth muscle cells. The hollow fibers were

constructed of cellulose, and thus the cells, which were placed on the outside of the fibers, degraded the fibers, leaving spaces for nutrient (blood) flow. He reported that the smooth muscle cells in this system worked, but the endothelial cells did not survive.

Dr. Tsutsumi's research addresses biomechanical problems associated with dental implant failure and uses biomechanical data (stress/strain measurements in animals subjected to an applied force) and finite element analyses to design tissue-engineered solutions. Artificial tooth roots are widely used in dental surgery to replace the lost function of natural teeth. They are fixed directly into the jawbone and thus the stresses and strains around the implant impact bone formation and resorption. In many cases, this leads to net bone resorption, loosening of the implant-bone interface, and ultimately to implant failure. Natural tooth roots are covered with a periodontal ligament (PDL) which is made up of fibroblasts and collagen fibers. The PDL acts as a shock absorber during mastication and as a receptor of forces. To increase the biocompatibility of the titanium surface of dental implants and the bone regenerating capacity around titanium dental implants, Dr. Tsutsumi's lab has coated implants with poly (ethylene- co-vinyl alcohol) (EVA), which has a high affinity for metal and which possesses good mechanical properties. EVA films were exposed to ozone to introduce carboxyl groups, and Type I collagen was immobilized onto the surface via a polyion complex. Human PDL cells were grown on the EVA surface with and without collagen. In the presence of collagen, good proliferation of cells was observed compared to EVA without collagen. The group expects that cultured PDL cells on collagen-coated EVA will lead to regeneration of the periodontal ligament, which could be used on artificial tooth implants to replace the properties of the natural tooth root PDL and ultimately lead to improved clinical durability.

Dr. Tomita described studies on total knee regeneration using internal and external fixators and a more novel magnetic fixator. When living bone is fixed with a rigid metal plate, problems such as local osteoporosis can occur. Dr. Tomita found that if a sliding motion is applied either to an external or an internal fixator, the regeneration of cartilage and overall articular structure was greater. So, the mechanical environment was found to be very important. Dr. Tomita also looked at the effects of static magnetic fields on bone formation in rat femurs. He is using tapered rods made of magnetized and unmagnetized samarium cobalt that are implanted into the middle diaphysis of rat femurs under press-fit loading. He found that the femurs adjacent to magnetized specimens had significantly higher bone mineral density and calcium content than those adjacent to the unmagnetized specimen. His results suggest that long-term local static magnetic field stimulation on the bone has a local effect to prevent the decrease in bone mineral density caused by surgical invasion or implantation.

LEGAL AND REGULATORY

The group indicated that the organ transplantation law states that human embryonic stem cells cannot be used for research purposes. Thus, they will not use ES cells in their research at this time. Within Kyoto University, use of human tissue is limited to hepatocytes for drug testing. The Kurokawa Committee established guidelines on use of human tissue in Japan; these state that only human tissue obtained within a university can be used for research at that university. For gene therapy experiments in humans, protocols must be approved by a university ethics committee. For clinical application, approval must be obtained from the Ministry of Education and the Ministry of Health and Welfare.

GOVERNMENT SUPPORT

The group outlined the four national projects in tissue engineering, as follows:

	Institution	Funding Agency
1.	Institute for Frontier Medical Science, Kyoto University	Ministry of Education
2.	Institute of Developmental Biologists (largest at 200 researchers. Will not do any regenerative medicine).	The Science and Technology Agency
3.	Tissue Engineering Center	Ministry of International Trade and Industry (MITI)
4.	Human Cell and Tissue Bank	Ministry of Health and Welfare

REFERENCES

Kyoto University. 1999. *Annual report of the frontier medical sciences Kyoto University*. Brochure.

—— 1999. Basic policy towards creation of biotechnology industry. Paper.

——— 1999. *Institute for frontier medical sciences*. Brochure.

Hirata, I., Y. Ikada, H. Iwata, H. Kitamura, E. Kitano, Y. Morimoto, and Y. Murakami. 2000. Study of complement activation on well-defined surfaces using surface plasmon resonance. *Colloids and Surfaces* 18:285-292.

Iwata, H., Y. Ikada, I. Ikai, H. Maeda, Y. Park, T. Sajiki, S. Satoh, T. Uesugi, and Y. Yamaoka. 1999. In vitro evaluation of metabolic functions of a bioartificial liver. *Asaio Journal* **45** (4):299-306.

Iwata, H., Y. Murakami, and Y. Ikada. 1999. Control of complement activities for immunoisolation. *Bioartificial Organs II* 875:7-23.

Kudo, S., Y. Hiraki, H. Mizuta, Y. Otsuka, and K. Takagi. 2000. Inhibition of chondrogenesis by parathyroid hormone in vivo during repair of full-thickness defects of articular cartilage. *Journal of Bone and Mineral Research* 15 (2):253-260.

Kudo, S., H. Mizuta, Y. Otsuka, K. Takagi, and Y. Hiraki. 2000. Inhibition of chondrogenesis by parathyroid hormone *in vivo* during repair of full-thickness defects of articular cartilage. *Journal of Bone and Mineral Research* 15:253-260.

Oowaki, H., N. Hashimoto, Y. Ikada, H. Iwata, S. Matsuda, T. Ohta, A. Sadato, N. Sakai, and W. Taki. 2000. Non-adhesive cyanoacrylate as an embolic material for endovascular neurosurgery. *Biomaterials* 1039-1046.

Otsuka, Y., Y. Hiraki, K-I Iyama, H. Mizuta, K. Nishikawa, F. Suzuki, K. Takagi, and Y. Yoshitake. 1997. Requirement of fibroblast growth factor signaling for regeneration of epiphyseal morphology in rabbit full-thickness defects of articular cartilage. *Development Growth Differ*. 39:143-156.

Sajiki, T., S. Fujita, Y. Ikada, I. Ikai, H. Iwata, H. Paek, Y. Park, S. Satoh, T. Tosha, Y. Ueda, Y. Yamaoka, and B. Zhu. 2000. Morphologic studies of hepatocytes entrapped in hollow fibers of a bioartificial liver. *Asaio Journal* 46 (1): 49-55.

Shimizu, Y. n.d. Outline of Study. Kyoto University Department of Bioartificial Organs. Paper.

———. 1999. Theory of site in bio-artificial organs that induce autologous tissue regeneration. Kyoto University Department of Bio-Artificial Organs Paper.

———. n.d. Theory of site in bio-artificial organs that induce autologous tissue regeneration. Website printout from http://www.frontier.kyoto- u.ac.jp/ca04/personal/shimizu/paper99_e.html.

Shukunami, C., T. Atsumi, Y. Hiraki, K. Ishizeki, C. Shigeno, and F. Suzuki. 1996. Chondrogenic differentiation of clonal mouse embryonic cell lines atdc5 in vitro: differentiation-dependent gene expression of parathyroid hormone (pth)/pth-related peptide receptor. *The Journal of Cell Biology* 133 (2):457-468.

Shukunami, C., T. Atsumi,, Y. Hiaki, K. Ishizeki, Y. Ohta, and F. Suzuki. 1997. Cellular hypertrophy and calcification of embryonal carcinoma-derived chondrogenic cell line atdc5 in vitro. *Journal of Bone and Mineral Research* 12 (8):1174-1188.

Tabata, Y. 2000. The importance of drug delivery systems in tissue engineering. *Pharmaceutical Science and Technology Today* 3 (3):80-89.

Site: **Kyushu University**
Graduate School of Medicine
Department of Biomedical Engineering
3-1-1 Maidashi, Higashiju
Fukuoka 812-8582 JAPAN

Date visited: 25 August 2000

WTEC Attendees: M. Mrksich (report author), H. Greisler, G. Holdridge, L. McIntire, N. Parenteau

Hosts: Professor Takehisa Matsuda
Tel.: 81-92-642-6210; Fax: 81-92-642-6212;
Email: matsuda@med.kyushu-u.ac.jp
Assistant Professor Toshinobu Sajiki, PhD
Tel.: 81-92-642-6211; Email: jsajiki@medeng.med.kyushu-u.ac.jp

SUMMARY

Professor Matsuda directs a research group in the Departments of Medical Engineering at Kyushu University and of Biomedical Engineering at the National Cardiovascular Center. Professor Matsuda has a technical background in the areas of chemistry and materials science, and his current programs in biomaterials and bioengineering are characterized by a strong position in these areas. His laboratory has developed new photochemical strategies for surface modification and a new class of photoreactive biomaterials. They have applied these materials and methods to fundamental studies of protein-substrate interactions, cell behavior, and the influences of cell shape on behavior. In a second theme, his group has developed photochemical routes to scaffolds that are being used in cardiovascular tissue engineering and cartilage tissue engineering. His research team at both sites comprises one assistant professor (Dr. Sajiki), one lab head (Dr. Nakayama), one researcher (Dr. Ohya), four postdoctoral associates, six MD graduate students, and four technicians. The annual budget is approximately $1.2 million USD, and is provided by the Welfare and Health Ministry (70%) and a Millenium Project (from Japanese Science and Technology Agency).

R&D ACTIVITIES

Biomaterial Design and Surface Process Engineering

This group has developed a range of photochemical methods for the preparation of synthetic polymers and the modification of the polymers with ligands. In a prototypical example, gelatin was modified with styrene units, which could then be cross-linked to give gels or modified with methacrylated heparin to generate grafts. A significant strength in this group is the range of methods for photoprocessing of the polymers. Chemistries have been developed that operate with light sources spanning from UV to IR in a variety of modalities (derivatization, grafting, layering, and lamination) and with processing strategies that include stereolithography, oblation, and cutting. These methods have been used to photolithographically pattern polymers on substrates, for the purpose of patterning the shapes, sizes, and positions of adherent cells. Examples were shown wherein endothelial cells remained patterned for periods of many weeks. The group used these patterned substrates to investigate a number of fundamental aspects, including actin alignment in adherent cells, anisotropic properties of aligned cytoskeletons (using atomic force microscopies), and various metabolic consequences of engineering cell shape.

Photochemical Scaffold Design

This group has developed several impressive routes to preparing three-dimensional polymer scaffolds. The capabilities include the use of excimer laser ablation to generate meshes, stereolithographies to generate microarchitectures, and photopolymerization routes to give fibers, meshes, and tubing. The group has focused on developing artificial vascular grafts made from polyurethanes. The basic approach uses laser

ablation to generate pores in the tubular constructs; the method is general in permitting a wide choice of pore diameter and spacing between pores. The methods also allow for the surface to be modified with VEGF, FGF, and other protein factors. The group has developed a new design based on two coaxial tubes to better reproduce the pressure-diameter relationship of native arteries. They are currently evaluating these grafts in a canine carotid artery model. A related effort is investigating new approaches towards metal stents that are modified with heparin-immobilized gels.

Tissue Engineering

The Matsuda scientists have applied the materials advances to cardiovascular and cartilage tissue engineering. In the former, they have used a three-layered structure containing endothelial cells, smooth muscle cells, and fibroblasts in a concentric arrangement (the Bell Model). In one modification, the fibroblasts have been genetically engineered to express VEGF, CNP, and TFPI as an alternative cell source to express temporally antithrombogenicity and to enhance tissue regeneration. In the cartilage project, they are developing injectable tissues based on chondrocyte immobilized in thermo-responsive gelatin and hyaluronic acid gels. The focus of this work has been on the fabrication of the construct, but work to evaluate the *in vivo* performance of the constructs has started.

INITIATIVE FOR TECHNOLOGY TRANSFER

Kyushu University recently established a Technology Licensing Office to serve faculty in patenting and licensing scientific discoveries. While this initiative represents the growing awareness in Japan that commercialization of university-derived research will have many economic benefits, the current infrastructure is still inadequate for reaching these goals. The initial budget for this office, for example, is ¥10 million (much of it contributed by the faculty) and cannot serve the needs of a university having 300 faculty members. Rather, faculty must still approach large corporations to adopt the technologies and to finance patents and commercialization. A consequence of this default position is that the technologies rarely can be developed in small start-up companies, which can accept the high degree of risk in emerging technologies.

SUMMARY

The Matsuda Group has a leading technical position in the development and use of photochemical strategies for materials design and fabrication. This technology is well suited for progress in tissue-engineering programs, and the group has made an excellent start in this direction. The further integration of cell biologists and clinicians into this team would result in a leading tissue-engineering effort. The procedures and support for technology transfer and commercialization are still underdeveloped and remain an obstacle to the maturation of tissue engineering.

REFERENCES

Defife, K., J. Anderson, E. Colton, Y. Nakamaya, and T. Matsuda. 1999. Spatial regulation and surface chemistry control of monocyte/macrophage adhesion and foreign body giant cell formation by photochemically micropatterned surfaces. 148-154.

Doi, K., and T. Matsuda. 1997a. Enhanced vascularization in a microporous polyurethane graft impregnated with basic fibroblast growth factor and heparin. *Journal of Biomedical Materials Research* 34:361-370.

———. 1997b. Significance of porosity and compliance of microporous, polyurethane-based microarterial vessel on neoarterial wall regeneration. 573-584.

Higashi, J., E. Marchant, T. Matsuda, and Y. Nakayama. 1999. High-spatioresolved microarchitectural surface prepared by photograft copolymerization using dithiocarbamate: surface preparation and cellular responses. *Langmuir* 15 (6):2080-2088.

Lee, H., Y. Nakayama, and T. Matsuda. 1999. Spatio-resolved, macromolecular architectural surface: highly branched graft polymer via photochemically driven quasiliving polymerization technique. *Macromolecules* 32 (21):6989-6995.

Masuda, S., K. Doi, T. Matsuda, T. Oka, and S. Satoh. 1997. Vascular endothelial growth factor enhances vascularization in microporous small caliber polyurethane grafts. *Asaio Journal* 43 (5): m530-m534.

Matsuda, T., S. Arnold, and M. Mizutani. 2000. Molecular design of photocurable liquid biodegradable copolymers. 1. synthesis and photocuring characteristics. *Macromolecules* 33 (3):795-800.

Matsuda, T., and M. Mizutani. 2000. Molecular design of photocurable liquid biodegradable copolymers. 2. synthesis of coumarin-derivatized oligo(methacrylate)s and photocuring. *Macromolecules* 33 (3):791-794.

Matsuda, T., and Y. Nakayama. 1996. Surface microarchitectural design in biomedical application: in vitro transmural endothelialization on microporous segmented polyurethane films fabricated using an excimer laser. *Journal of Biomedical Materials Research* 31:235-242.

Matsuda, T., N. Saito, and T. Sugawara. 1996. Ceric-ion-initiating surface graft polymerization with regional control and dimensional precision. 1996. *Macromolecules* 29 (23):7446-7451.

Matsuda, T., and T. Sugawara. 1995. Development of a novel protein fixation method with micron-order precision. *Langmuir* 11 (6):2267-2271.

———. 1995. Development of surface photochemical modification method for micropatterning of cultured cells. *Journal of Biomedical Materials Research* 29:749-756.

———. 1995. Photochemical protein fixation on polymer surfaces via derivated phenyl azido group. *Langmui*r 11 (6):2272-2276.

Morioka, M. 1995. Bioethics and Japanese Culture: Brain death, patient's rights, cultural factors. *Eubios Journal of Asian and International Bioethics* 5:87-90.

Morioka, M. 2000. Commentary on seewald. *Eubios Journal of Asian and International Bioethics* 10:76-77.

Nakamaya, Y., K. Goto, Y. Hirano, T. Matsuda, and M. Miamura. 1999. Preparation of poly(ethylene glycol)-polystyrene block copolymers using photochemistry of dithiocarbamate as a reduced cell-adhesive coating material. *Biomaterials* 963-970.

Nakamaya, Y., K. Goto, Y. Hirano, K. Nakamata, and T. Matsuda. 1998. Surface hydrogelation of thiolated water-soluble copolymers on gold. *Langmuir* 14 (14):3909-3915.

Nakamaya, Y., and T. Matsuda. 1999. In situ observation of dithiocarbamate-based surface photgraft copolymerization using quartz crystal microbalance. *Macromolecules.* 32(16), 5405-5410.

———. 1996. Surface macromolecular architectural designs using photo-graft copolymerization based on photochemistry of benzyl n,n-diethyldithiocarbamate. *Macromolecules*. 29(27), 8622-8630.

———. 1999. Surface macromolecular microarchitecture design: Biocompatible surfaces via photo-block-graf-copolymerization using n, n-diethyldithiocarbamate. *Langmuir* 15 (17):5560-5566.

———. 1995. Surface microarchitectural design in biomedical applications: Preparation of microporous polymer surfaces by an excimer laser ablation technique. *Journal of Biomedical Materials Research* 29:1295-1301.

Seewald, R. 2000. A survey of the attitudes of 252 Japanese nurses toward organ transplantation and brain death. *Eubios Journal of Asian and International Bioethic.* 10:72-76.

Sugawara, T., and T. Matsuda. 1995. Photochemical surface derivatization of a peptide containing arg-gly-asp (rgd). *Journal of Biomedical Materials Research* 29:1047-1052.

———. 1996. Synthesis of phenylazido-derivatized substances and photochemical surface modification to immobilize functional groups. *Journal of Biomedical Materials Research* 32:157-164.

Ziani-Cherif, H., K. Imachi, and T. Matsuda. 1999. Preparation of aryldiazonium-, aryldiazo-, and arylazido-derivatized copolymers and their surface photografting. *Macromolecules* 32 (10):3438-3447.

Site: **Nippon Telegraph and Telephone Corporation (NTT)**
Basic Research Laboratories
Atsugi R&D Center
3-1 Morinosatao Wakamiya
Atsugi-Shi, Kanagawa, Japan
http://www.brl.ntt.co.jp

Date visited: 24 August 2000

WTEC Attendees: N.L. Parenteau (report author), H. Greisler, L. McIntire, G. Holdridge, M. Mrksich

Hosts: Dr. Keiichi Torimitsu, Group Leader, Molecular and Bio-Science Research Group, Materials Science Laboratory, Tel: +81-46-240-3562; Fax: +81-46-270-2364, 250-7993; Email: torimitu@will.brl.ntt.co.jp
Dr. Yasuhiko Jimbo, Senior Research Scientist, Molecular and Bio-Science Research Group, Email: jimbo@will.brl.ntt.co.jp

BACKGROUND

NTT Basic Research Laboratories promotes research activities to contribute to scientific knowledge and establish basic technologies. Its goal is to provide "useful research products" to serve the public, industry, and academia. Since it was once a government organization and the government is still a major shareholder, Dr. Torimitsu explained that it is the duty of the labs to give something back to the public in the form of useful basic technology. It is operated more as an open basic research laboratory than as a company laboratory. Its mission is to find new concepts and develop knowledge in the areas of device physics, materials science, quantum optics/optical materials, and quantum electron physics. Our hosts provided two booklets and other information outlining their structure and research activities throughout the organization.

The organization of NTT basic research laboratories has changed recently, becoming more simplified. NTT Basic Research Laboratories are headed by Dr. Sunao Ishihara, whom we met briefly. Dr. Ishihara was well aware of WTEC efforts in the nanotechnology area. He commented that the United States has stimulated the Japanese to set in motion their initiatives in nanotechnology to remain competitive. The operation is divided into 4 divisions: Research Planning, Device Physics Laboratory, Physical Science Laboratory, and the Materials Science Laboratory. Dr. Hideaki Takayanagi, Executive Manager, whom we met briefly, heads the Materials Science Laboratory. The laboratory is divided into 4 groups:

1. Molecular and Bio-Science Research Group
2. Superconducting Thin Films Research Group
3. Superconducting Quantum Physics Research Group
4. Nano-Structure Materials Research Group

Tissue engineering or biological applications are limited to the Molecular and Bio-Science Research Group, of which Dr. Torimitsu and Dr. Jimbo are members.

Group Overview

Dr. Torimitsu presented an overview of the Molecular and Bio-Science Group research projects that he felt were most relevant to our interests. NTT has about 3000 researchers. Total R&D expenditure is ¥ 200 billion (¥ 200,000 million), including salaries. NTT spends 5% of this for basic research. The group is funded at a level of 1-2% of this basic research expenditure, and consists of 9 PhDs, 1 master's-level researcher, and 1-2 postdoctoral fellows.

R&D ACTIVITIES

Surface Modification of Materials

Dr. Torimitsu's researchers are using silicone compounds, modified polysilanes, to modify surface properties of materials. They are able to cut the polysilanes and modify one end to attach to a surface. These bound polysilanes can be modified using different side chains to achieve different shapes. They are able to determine specifically where the chain should attach. Using these shape changes, they can modify the electrical conductivity of a surface, optical energy in response to heat, etc. These structures have memory: for example, a helical structure can change in response to heat or optical energy then return to the helical structure once the energy is removed.

Planar Electrode Arrays

The group's work involved the use of planar electrode arrays to measure neuronal networks, both in brain slices and neuronal cell cultures. Our hosts provided reprints of two papers covering these topics, Jimbo and Robinson 2000; Jimbo et al. 1999. The latter paper was later described by Dr. Jimbo as the group's most interesting result in this field: "Its significance is that plasticity in a group of neurons (not in the level of synaptic phenomena) and its governing rule was obtained. Without taking advantage of multisite recording, this kind of recording was impossible."

The planar electrode array is able to simultaneously record neuronal activity. Electrode arrays are fabricated by photolithography using a quartz substrate sputter coated with indium tin oxide (ITO). The ITO layer is wet-etched to form the electrode patterns and the surface insulated with a silicon-based photoresist. The insulation is selectively removed at electrode terminals using reactive ion etching. The array is comprised of 64 electrodes arranged in two areas separated by 500 microns, each containing a 4X8 square grid. The size of the recording terminals is 30 X 30 μm, and the distance between adjacent terminals is 150 microns. The surfaces of the recording terminals are electrochemically coated with a thin layer of platinum black to reduce interface impedance (100 kΩ at 1 kHz).

Previously, members of the group have used the electrode array with cell substrates fabricated with 1 micron grooves in an attempt to form ordered circuits, the neurons following the grooves (Hirono et al. 1988, Torimitsu et al. 1990, Jimbo et al. 1993). This ordered culture can be maintained for about two to three weeks, but eventually other cell types grow beyond the grooves and make random contacts.

This system appears elegant and highly sensitive. Electrical activity changes based on number of synaptic inputs. The group is also able to monitor change in signal direction. Going beyond electrical activity, these researchers have developed a glutamate-specific sensor to measure synaptic activity using an l-glutamate oxidase film which converts l-glutamate to alpha-ketoglutarate, which potentiates a current on the electrode surface (Torimitsu and Niwa 1997). They are currently studying the effects of neurotrophic factors such as nerve growth factor on electrical activity (Torimitsu et al. 1999, Torimitsu et al. 2000). Using this system, they are able to measure electrical activity, neurochemical response, and ions (via multi-photon laser microscope and RAMAN laser spectroscopy) in response to neurotrophic factors. The system could be used to record the activity of living networks over long periods of time. Dr. Jimbo provided a demonstration of electrical output from a neuronal cell culture in real time. Activity could be constantly monitored over many days. Dr. Torimitsu's group's long-term vision is that this system could have a medical application in the development of implantable electrodes to measure brain activity after stroke, for example, or possibly to restore neural function.

The laboratory was superbly equipped with state-of-the-art instruments. This included an atomic force microscope, scanning electron microscope, multi-photon laser microscope, and RAMAN laser spectroscopy.

PARADIGM SHIFTS

A paradigm shift for NTT is the use of their researchers' skills for biological problems. The team, consisting of two electrical engineers, one electrical chemist, and one optical physicist, is well funded and well

positioned for leadership in the area of neural networks, biological sensor interfaces, etc. It is noted that the group at present lacks a biologist, which would further enhance the competitiveness of the team.

REFERENCES

Ajito, K., and K. Torimitsu. 2001. Near-infrared Raman spectroscopy of single particles. *Trends in Anal. Chem.* 20:255-262.

Hirono, T., K. Torimitsu, A. Kawana, and J. Fukuda. 1988. Recognition of artificial microstructures by sensory nerve fibers in culture. *Brain Research* 446:189-194.

Jimbo, Y., I. Kulagina, S. Korogod, and K. Torimitsu. 2000. Spatio-temporal patterns of action potentials in a single neuron. Forum of European Neurosci. 2000. *Eur.. J. Neurosci.* 12, Suppl.11:383.

Jimbo, Y., and H.P.C. Robinson. 2000. Propagation of spontaneous synchronized activity in cortical slice cultures recorded by planar electrode arrays. *Bioelectrochemistry* 51:107-115.

Jimbo, Y., H. Robinson, and A. Kawana. 1993. Simultaneous measurement of intracellular calcium and electrical activity from patterned neural networks in culture, *IEEE Trans. BME*-40, pp. 804-810.

Jimbo, Y., T. Tateno, and H.P.C. Robinson. 1999. Simultaneous induction of pathway-specific potentation and depression in networks of cortical neurons. *Biophysical Journal* 76:670-678.

Jimbo, Y., and K. Torimitsu. 1999. Modification of evoked responses in cultured cortical networks. BMES & EMBS (21st Ann. Int. Conf. Eng. Med. Biol. Soc.) 4.1:2-4

———. 2000. Multi-Site recording of neural activity: Spatio-temporal patterns of action potentials in a single neuron. *Tech. Report IEICE* 99 (1-5):114.

Kasai, N., Y. Jimbo, O. Niwa, T. Matsue, and K. Torimitsu. 2001. Real-time multisite observation of glutamate release in rat hippocampal slices. *Neurosci. Lett.* 304:112-116.

Nakashima, H., J. Koe, K. Torimitsu, and M. Fujiki. 2001. Transfer and amplification of chiral molecular information to polysilylene aggregates. *J. Am. Chem. Soc.* 123:4847-4848.

Nippon Telegraph and Telephone Corporation. 1999. Research activities in NTT basic research laboratories. *Nippon Telegraph and Telephone Corporation (NTT) Basic Research Laboratories* 10:1-39.

Niwa, O., R. Kurita, T. Horiuchi, and K. Torimitsu. 1998. Small-volume on-line sensor for continuous measurement of g–aminobutric acid. *Anal. Chem.* 70:89-93.

Robinson, H.P.C., M. Kawahara, Y. Jimbo, K. Torimitsu, Y. Kuroda, and A. Kawana. 1993. Periodic synchronized bursting and intracellular calcium transients elicited by low magnesium in cultured cortical neurons. *J. Neurophysiol.* 70:1606-1616.

Torimitsu, K., Y. Furukawa, N. Kasai, and Y. Jimbo. 2000. Effect of neurotrophic factors on neural activity in cultured rat cortex. In T. Kato ed. *Frontiers of the Mechanisms of Memory and Dementia*. Elsevier Science B.V. 173-176.

Torimitsu, K., and A. Kawana. 1990. Selective growth of sensory nerve fibers on metal oxide pattern in culture. *Dev. Brain Res.* 51:128-131.

Torimitsu, K., K. Kurita, and O. Niwa. 1999. Enzyme-based real-time detection of synaptic glutamtate release modulated by GABA and neurotrophins in cultured rat neurons. In K. Uyemura, K. Kawamura, and T. Yazaki, eds. *Neural Development,* 474-480. Tokyo: Springer-Verlag..

Torimitsu, K., and O. Niwa. 1997. Real-time detection of GABA-induced synaptic glutamate release in cultured rat cortex. *NeuroReport 8* 1353-1358.

Site: **Nagoya University**
Graduate School of Medicine
Department of Oral and Maxillofacial Surgery
65 Tsurumai-Cho, Showa-Ku
Nagoya, 466-8550 Japan

Date visited: 23 August 2000

WTEC Attendees: D. Mooney (report author), D. Smith, A. Russell, C. Kelley, H. Morishita

Hosts: Minoru Ueda, DDS, PhD, Professor and Chairman, Tel: 81-52-744-2345; Fax: 81-52-744-2352; Email: mueda@tsuru.med.nagoya-u.ac.jp

SUMMARY

This was an informal meeting in Dr. Ueda's office, with no formal presentations from either our host or the visiting WTEC group. No other members of his laboratory attended. The meeting consisted of an informal discussion around the state of tissue engineering in Japan. Dr. Ueda has been working with Howard Green's epithelial sheet approach to skin replacement for approximately 20 years and has treated approximately 100 patients with this engineered tissue. He is working with a variety of other tissues as well. His focus is tissue regeneration applied to the head and neck, not organ engineering. He is one of the key people behind the founding of Japanese Tissue Engineering Company (J-TEC), which our group visited the same day.

TISSUE ENGINEERING INDUSTRY IN JAPAN

Dr. Ueda believes that the level of academic activity in tissue engineering in Japan is similar to that in the United States, but that the development of a tissue engineering industry is ocurring at a much slower pace. He believes there are several underlying reasons for this:

1. Most Japanese researchers in biotechnology are in the national universities, and it is not easy for these researchers to cooperate with industry.
2. Japanese companies do not like to risk going into new areas.
3. The government is an impediment, due to its slowness in addressing new issues and the regulatory burden in Japan. He cited as an example that he served on a committee responsible for suggesting regulatory guidelines in tissue engineering to the Ministry of Health and Welfare. The guidelines were finished over a year ago, but the Ministry has yet to act on the draft.

Dr. Ueda believes many universities are laying the groundwork for starting companies, and he expects the formation of 10 tissue engineering companies in Japan by 2003. He also expects commercial activity in Singapore, Hong Kong, Taiwan and other Asian countries.

KEY TO SUCCESS IN TISSUE ENGINEERING

Dr. Ueda heavily stressed the importance of two issues in achieving success in the tissue-engineering field. First, he stressed that the effort must be led by a clinician, due to need for knowledge of the clinical issues. Secondly, a highly interdisciplinary approach is required, with the clinician providing guidance to the other scientists in terms of direction.

R&D ACTIVITIES

Biomaterials

There is no overarching philosophy behind the choice of biomaterials for his tissue engineering applications. Dr. Ueda is interested in using whatever material is shown to work in a specific application. His group has mainly focused on using collagen-based scaffolds in its work to date, but members also work with synthetic polymers and ceramics.

Biomolecules

Dr. Ueda's group does not have an interest in this approach to tissue engineering, and he believes this approach is just starting in Japan. His group has performed some studies using *ex vivo* genetic modification of cells in skin tissues to produce therapeutic proteins. However, he believes genetic engineering approaches to tissue engineering are poor candidates for a business in Japan, due to the strict regulation by the Japanese government.

Cells

The main focus of Dr. Ueda's work has been autologous cells for skin engineering, but he now has a significant interest in other autologous cell types as well. These include cells for peripheral nerve regeneration, cartilage regeneration, mesenchymal stem cells, and corneal epithelial cells. He believes a critical step in the development of tissue engineering in Japan is the formation of a cell-processing center that can proliferate cells and make them available to researchers and companies in Japan. This center was planned to open in the Kansai area in 2001. He foresees this center will handle stem cells isolated from cord blood, bone marrow-derived stem cells, and someday, stem cells from all tissue types. The initial focus of this facility will be autologous cells, but it will eventually cover allogeneic cells as well. The concept of a cell bank is starting on a small scale in Dr. Ueda's lab, as Nagoya University has approved for him to provide cells to J-TEC, which will then sell its cell-based product.

A major interest of Dr. Ueda's is embryonic stem (ES) cells, as he believes they will play a critical role in the development of tissue-engineered products. He believes that there are many fewer ethical and social hurdles to the use of ES cells in Asian cultures than in Western cultures, and this will provide a key advantage to Japanese companies in the tissue engineering area. He believes many Asian investigators see ES cell-based products as the key to overcoming the current U.S. lead in the genome sciences. He and other researchers at universities in Japan are publicly prevented from pursuing this line of research currently. However, he is working with animal ES cells, and he has collaborators in China who are adapting the concepts he works out with animal cells to human ES cells. An important distinction he drew in Japanese ES cell research is that while he and other investigators cannot publicly work with these cells (e.g., publish or present papers at meetings), they can informally work with these cells and collect data. He believes many researchers in Japan are currently taking this approach in preparation for the time when human ES cell research is allowed.

Biomechanics

Dr. Ueda's lab is performing studies in 2D cell culture in which mesenchymal stem cells are subjected to mechanical strain in an effort to increase the percentage of cells that commit to a bone fate.

REGULATORY ISSUES

Dr. Ueda believes Japanese regulations of tissue-engineering products will follow the lead of the FDA in the United States. In some cases, he expects autologous cell products to be regulated if the cells have been cultured.

Site: **National Cancer Center Research Institute**
1-1, Tsukiji 5-chome
Chuo-ku, Tokyo 104-0045 Japan

Date visited: 21 August 2000

WTEC Attendees: L.V. McIntire (report author), H. Griesler, N. Parenteau, G. Holdridge

Host: Dr. Takahiro Ochiya, Section for Studies on Matastasis
Tel: 81- 3-3547-5237; Fax: 81-3-3541-2685; Email: tochiya@ncc.go.jp

BACKGROUND

The National Cancer Center Research Institute (NCCRI) is a large government research facility adjacent to a very large cancer treatment hospital in Tokyo. The NCCRI is the main research institute in the country dedicated to cancer research and is tightly coupled to the hospital.

We talked briefly with Dr. Setsuo Hirohashi, the Director of the NCCRI (shirohas@ncc.go.jp). He explained there was a large emphasis on the quality of science and the exact title of the division was not as important. Each division is supported at the level of approximately $800,000/year, excluding salaries. We discussed the Japanese Government Millennium Project initiated in 2000: tissue engineering (reparative biology) and gene therapy are focus areas. Dr. Hirohashi gave an overview of this. Tthe main center for tissue engineering for this project is in the Kyoto-Kobe area. There is a new emphasis on the interface with industry. It is very positive to obtain patents in these new projects, but obtaining funds to pay for this process has been problematic in NCCRI.

R&D ACTIVITIES

Dr. Ochiya's group has several research interests, a major one being cancer gene therapy. His approach has been to develop plasmid DNA delivery systems for controlled release and long-term gene expression. His researchers incorporate DNA vectors (or antisense oligonucleotides) into biomaterials for extending DNA lifetimes. The biomaterial has been atelocollagen—pepsin digested tropocollagen from Kogen Biotechnology (although Sumitomo has the patent for the material). This collagen is soluble at low temperature but forms a hard material at body temperature. It can also be manufactured as a cylindrical mini-pellet and stored for several months at 4°C. They incorporate the plasmid DNA with the collagen, then inject or insert pellets. The studies have been done mainly with an FGF-4 (HST-1) gene and adenovirus construct. FGF-4 expression leads to increased platelet production.

In vivo studies were done in nude mice with intramuscular injection of pellets. They used PCR for monitoring plasmid DNA release and ELISA for FGF-4 protein production. They found DNA release and protein production for over 60 days—with little inflammatory response to the pellet (though there was some fibrosis). 30% glucose was added to the final collagen mixture to improve release. By delivery of FGF-4, they were able to rescue mice from normally fatal (9 Gray) radiation treatment exposure. They hope to use this eventually in humans to help after chemotherapy or irradiation treatments in cancer patients. There are other effects of FGF-4, but these are still under investigation. Incorporation in atelocollagen not only increases DNA stability and prolongs release but also reduces the immunogenicity of the adenovirus construct, which allows repeat-administration of adenovirus vectors. The collagen can also be manufactured in the form of nanoparticles for direct injection in the bloodstream. Dr. Ochiya is also interested in DNA vaccine development for hepatitis B and C through atelocollagen implant.

A second area of research is the use of murine embryonic stem cells (ES) for development of new blood vessels or development into hepatic lineage. For vascular cells, culture is done in hyaluronic acid gels with activin A. For monitoring hepatic cell development, a GFP reporter gene attached to the albumin promoter has been used. Dr. Ochiya is also trying to develop a rat ES model; this would be very important for generating KO rat models for cancer research.

Biomaterials

Emphasis is on use of atelocollagen for DNA delivery. There are certainly applications for tissue engineering and Dr. Ochiya is very aware of them; however, his main interests are in cancer applications.

Biomolecules

Use of FGF-4 seems novel. Apparently the molecule (HST-1) was cloned from 3T3 cells at the NCCRI.

Cells

NCCRI researchers are using murine embryonic stem cells in developmental models for vascular and hepatic tissues. They are trying to develop a rat ES model.

Bioinformatics

Bioinformatics is not an emphasis in this group, although a large Cancer Genomics Project has recently been initiated, and a new facility for this center is being constructed on the NCCRI site.

REGULATORY ISSUES

Human trials are not being done at present. Human ES use is restricted by the Japanese government. Patent involvement is just beginning.

REFERENCES

National Cancer Center Research Institute. 1999. Annual Report. Catalog.

_____1999. Cancer statistics in Japan. Catalog.

Ochiya, T., and M. Terada. 1999. Antisense approaches to in vitro organ culture. *Methods in Enzymology* 314:401- 411.

Ochiya, T., S. Nagahara, A. Sano, H. Itoh, and M. Terada. 2001. Biomaterials for gene delivery: Atelocollagen-mediated controlled release of molecular medicines. *Current Gene Therapy* 1 (1):31-52.

Ochiya, T., Y. Takahama, S. Nagahara, Y. Sumita, A. Hisada, H. Itoh, Y. Nagai, and M. Terada. 1999. New delivery system for plasmid DNA in vivo using atelocollagen as a carrier material: The minipellet. *Nature Medicine* 5 (6):707-710.

Quinn, G., T. Ochiya, M. Terada, and T. Yoshida. 2000. Mouse fit-1 promoter directs endothelial-specific expression in the embyroid body model of embryogenesis. *Biochem Biophys Res Commun.* 276 (3):1089-1099.

Sato, Y., T. Ochiya, Y. Yasuda, and M. Kenichi. 1994. A new three-dimensional culture system for hepatocytes using reticulated polyurethane. *American Association for the Study of Liver Diseases* 1023-1028.

Takahama, Y., T. Ochiya, H. Sasaki, H. Baba-Toriyama, H. Konishi, H. Nakano, and M. Terada. 1998. Molecular cloning and functional analysis of cDNA encoding a rat leukemia inhibitory factor: towards generation of pluripotent rat embryonic stem cells. *Oncogene* 16:3189-3196.

Yamamoto, H., T. Ochiya, Y. Takahama, Y. Ishii, N. Osumi, H. Sakamoto and M. Terada. 2000. Detection of spatial localization of *Hst-1/Fgf-4* gene expression in brain and testis from adult mice. *Oncogene* 19:3805-3810.

Site: **National Institute for Advanced Interdisciplinary Research (NAIR)**
1-1-4 Higashi, Tsukuba
Ibaraki 305-8562 JAPAN
http://www.aist.go.jp/NAIR/

Date visited: 23 August 2000

WTEC Attendees: N.L. Parenteau (report author), L. McIntire, M. Mrksich, H. Greisler, and G. Holdridge

Hosts: Jun Miyake, PhD, Chief Senior Researcher, Director, Biotechnology Group, Tel: +81-298-61-2558; Fax: +81-298-61-3009; Email: miyake@nair.go.jp
Hiroshi Watanabe, Director for Research Coordination and Planning, Email: hwatanabe@nair.go.jp

BACKGROUND

The Agency of Industrial Science and Technology (AIST) has several research institutes, one of which is the National Institute for Advanced Interdisciplinary Research (NAIR). NAIR is unique among AIST groups. It is not permanent but was established for a 7-year period. This institute disappears in 2001. This was an experiment. The institute has 50 permanent tenured PhDs and 1500 researchers overall. This is relatively small compared to the other AIST institutes, although it is very active and generates several times more publications than other institutes, making it tops among the AIST institutes according to Miyake. There is a new consolidated group beginning in April 2001 consisting of a large research institute and a small task force center for tissue engineering. The task force also has a 7-year commitment and will involve 10-20 tenured staff with a clear purpose to contribute to applied research to establish a basis for industry. NAIR is encouraged to have contact with industry but not with individual companies. There is an industrial deputy project leader who represents a company consortium. This person divides his time between the company and NAIR. The consortium system is unique to NAIR but will be extended to the new research center. The new center will be established in Osaka. The main purpose of NAIR is like that of NIST in the United States, to support companies to reach to new technologies. In fiscal year 2000 NAIR started a tissue-engineering project under the government Millennium project, which is for 5 years.

The Tissue Engineering Research Center (TERC) will develop technologies to support tissue engineering. Dr. Miyake will be the chief executive of the new center. The scientific leader will be Dr. Tateishi who is based at the University of Tokyo. The purpose will be to develop technologies to produce cell-based medical devices. The center's mission is to develop a new industry that will have an economic impact, to solve the shortage of human organs, to reduce the cost of medical care, to provide alternatives to animal testing, and to detect environmental toxins. It will have 22 domains or centers with 10-15 staff each. It is a research institute of AIST and MITI. Research is supported by MITI at $4.2 million per year. It will be housed in a new $30 million building of 4200 square meters by 2002. Activities will also include human cell culture at a cell-processing center. This center will be for basic research and will also serve as a common platform to organize and facilitate efforts of companies and researchers in the area of human cell culture. For example, it could serve as a central processing center for cultured cells used in clinical applications, such as bone and cartilage repair. The cell-processing center will interact with companies, researchers, and hospitals as a central, quality-controlled resource for autologous cell processing and eventually allogeneic cell processing. One of its first "products" will be processing autologous cartilage for chondrocyte implantation.

NAIR's intellectual property is handled by NEDO, a unit of AIST. NEDO has its own projects but is controlled by AIST. NEDO is private sector, which allows it to interact with companies to license technology. NAIR is public sector and therefore does not deal with licensing directly. The professors hold joint appointments with the University of Tokyo. This provides access to graduate students and yet allows the researcher to receive company funding.

R&D ACTIVITIES

The Bionic Design Group consists of 5 research teams involved in the following areas:

Soft tissue engineering. This team conducts R&D of artificial blood vessels, cultured blood vessels, artificial vitreous body, and hybrid type biomaterials. There was at least one research poster on an artificial blood vessel seen during our tour. A liver cell bioreactor based on a porous Teflon membrane and 3D culture of chondrocytes under hydrostatic pressure is highlighted in the brochure.

Hard tissue engineering; R&D on bone and cartilage. Chondrocyte culture was highlighted as an activity that would be performed at the new Cell Processing Center, which will be located in Osaka. Researchers are in pilot human clinical trials with both autologous bone and cartilage. It appears this involves the seeding of scaffolds with autologous cells. Our hosts did not present details of their methods.

RNA engineering. The electronic state of RNA is analyzed for molecular structure and function. This area was not covered, and no specific research was seen regarding this area during our tour.

Peptide engineering. This team conducts R&D of basic technology for constructing artificial molecular systems to mimic structures and functions. There was no discussion or comment concerning this area.

Molecular motor engineering. This team seeks to establish basic technology for constructing molecular machines based on muscle proteins.

This team is currently involved in research in the following areas:

- 3D cell culture scaffolds
- Genetic technologies and genomics
- Developmental and "differentiation" biology
- Stem cells and their application

Researchers are attempting to design novel scaffolds; the WTEC team saw a poster of an artificial vascular graft utilizing PTFE tube with a dense heparinized collagen layer on the lumen. They have experience with pressure-induced differentiation of cartilage. The cartilage and bone are in the clinic; the blood vessel is in animal trials.

PARADIGM SHIFTS

Funding Strategy and Technology-to-Market Issues

Dr. Miyake predicted that technology developed by the Cell Processing Center would most likely end up in large companies. He felt that the Japanese culture favored efforts in large companies, which was safer for the individual researcher than the more risky entrepreneurial route. However, Hiroshe Watanabe, a MITI representative whom the WTEC team met during this visit, countered this. It was Mr. Watanabe's expectation that entrepreneurship will be on the increase in Japan. He also remarked that the U.S. progress in tissue engineering was a "shock" to Japan and that they have recognized the need to be competitive in this area. He also remarked that unlike the past, MITI is presently quite open to foreign companies, no longer being focused solely on protecting Japan. There is government support for entrepreneurial activity in the form of tax relief. A company would be only taxed on half of its profit, with the other half reserved for future R&D. The scheme was developed 1-2 years ago. Japan is also establishing a system for small cap stocks similar to NASDAQ.

CONCLUSION

NAIR and the new Cell Processing Center are involved in a number of research areas and experimental approaches that are strikingly similar to past and current activities in U.S. laboratories. It appears that the first leg of their work will involve the implementation of what we consider to be comparable to current U.S. technologies. Although there was discussion of contributing novel approaches, it does not appear that they are yet to that stage. It should be noted, however, that they are in the clinic with bone and cartilage cells, and

with the new centralized cell processing center to enable and expedite culture of cells for clinical use, this group may be in a position to learn from the clinic and advance more quickly from that point compared to the United States, with its non-centralized process and regulation.

REFERENCES

National Institute for Advanced Interdisciplinary Research. n.d. National institute for advanced interdisciplinary research. Catalog..

_____ n.d. Tissue engineering research center. Brochure

_____ n.d.National institute of bioscience and human-technology. Brochure.

_____ 2000. Industrialization of tissue engineering. Brochure.

Site: **National Institute of Bioscience and Human Technology**
Agency of Industrial Science and Technology (AIST/MITI)
1-1 Higashi, Azuma, Tsukuba
Ibaraki 305-8566, Japan

Date visited: 22 August 2000

WTEC Attendees: A. Russell (report author), H. Greisler, G. Holdridge, C. Kelly, L. McIntire, D. Mooney, M. Mrksich, N. Parenteau, D. Smith

Hosts: Dr. Takashi Hirano, Head, Biopolymers Laboratory
Tel.: +81-298-61-6152; Fax: +81-298-61-6144; Email: thirano@nibh.go.jp
Dr. Youji Mitsui, Chief Senior Researcher
Email: ymitsui@nibh.go.jp
Dr. Kuriama, Head, Research Planning Office

INTRODUCTION

The National Institute of Bioscience and Human Technology (NIBHT) was established in 1993 under the auspices of MITI's Agency for Industrial Science and Technology (AIST) from a combination of 4 pre-existing institutes. In addition to administration staff, there are 50 research groups organized into 8 departments. Each of the research groups have 5-20 researchers. NIBHT also runs the Patented Organism Depository, which holds 13,000 strains. There are significant technology transfer activities at NIBHT. Indeed, in addition to 200 researchers, 40 administrators, 200 research assistants, and 200 post doctoral fellows, there are 70 corporate researchers on site at any given time.

NIBHT's annual budget, ¥3 billion in FY 2000 (~ $30 million), is supplied by AIST/MITI (80%), STA (10%), and the Patent Office (10%).

Current major focus areas are molecular and cellular biology; applied microbiology and bioengineering; neurosciences; and ergonomic and human technology. In April 2001, all AIST institutes were to be reorganized under a new name, tentatively the National Institute of Advanced Interdisciplinary Research. The new structure was expected to have 22 research centers, 22 labs, 9 special groups, and 2 research complexes to be located in the Kansai area. Of the new research centers, 5 will be bio-related:

1. Tissue Engineering Research Center (TERC)
2. Computational Biology Research Center
3. Structural and Functional Genomics Research Center
4. Gene Discovery Research Center
5. Quality of Life Research Center

TERC will be located in Kansai (Osaka area) after 2003, but will begin at NIBHT. Centers will have strong company involvement.

NIBHT will be divided up among the new centers, labs, and groups. Once formed, the TERC will receive 7 years of funding. Currently, funding is not linked to the results of evaluations of productivity.

A national MITI-funded tissue-engineering project was proposed in 2000, but the lack of commercial involvement and the long-term nature of the work prevented funding. Instead, $4 million was awarded for *basic* research at the National Advanced Interdisciplinary Research Institute (NAIR, also within MITI/AIST—see NAIR site report). Dr. Hirano's group at NIBHT (and its successor institutes) plan to propose a large applied tissue-engineering research project again after 2-3 years, with the expectation that companies will be involved.

The focus of the new TERC will likely be bone and cartilage engineering, with a sub-focus on biomaterials.

R&D ACTIVITIES

NIBHT is not working in the basic tissue-engineering field, but has significant expertise in a number of enabling technologies, such as drug delivery and DNA chips.

Biomaterials

Dr. Hirano is working on drug delivery for tissue engineering. His particular focus is on immobilization of super oxide dismutase to protect biological tissues. DIVEMA polymeric supports appear to have utility in stabilizing a number of enzymes and other proteins.

Cells

Dr. Mitsui, who is also a Professor at Tsukuba University, has worked for many years to develop an understanding of how to immortalize cells. The basic research program impacts our understanding of the aging process. Current work is focused on immortalization of vascular endothelial cells from human umbilical cord and on human embryonic stem cells. Dr. Mitsui has successfully induced immortalization by transfection of Mortalin (mot-1 and mot-2). The cells used were 3T3 cells. He has cloned human mot-2, which induces immortalization in the mouse, and inserted into human fibroblasts. There is a 30% increase in the number of achievable doublings. His group is now studying immortalization of islets and hepatocytes, with an interest in many immortalization-inducing genes. The immortalized fibroblasts appear normal and exhibit contact inhibition, thus they are *not* tumoragenic. The responsiveness of the cells to growth factors is subtly different to the normal mortal fibroblasts. Scale-up of the cell cultures is under way via collaboration, as are biomechanical interactions.

Biosensors

Glucose sensors are being developed.

REGULATORY ISSUES

Human immortalized cells are considered a very attractive alternative to ES cells, since in Japan, use of ES cells is problematic, culturally. Further, since the immortalization genes are natural, the government may not consider this gene therapy. If it is deemed gene therapy, the regulatory hurdles will be high and perhaps insurmountable. Our hosts commented that organ transplantation presents a problem in Japan due to religious sensitivities. However, the Ministry of Health and Welfare had just recently announced a new policy on this.

Site: **Institute of Physical and Chemical Research (RIKEN) Cell Bank**
3-1-1, Koyadai
Tsukuba Science City, 305-0074
Japan
http://www.rtc.riken.go.jp

Date visited: 22 August 2000

WTEC Attendees: L.V. McIntire (report author), H. Griesler, G. Holdridge, C. Kelly, D. Mooney, H. Morishita, N. Parenteau, A. Russell, D. Smith

Host: Dr. Tadao Ohno, Director, RIKEN Gene Bank
Tel:+81-298-36-9124; Fax: 81-298-61-6144; Email: tad-ohno@rtc.riken.go.jp

BACKGROUND

Dr. Ohno gave an introduction to RIKEN, making the analogy with the Max Plank Institutes in Germany. He is the director of the RIKEN Gene Bank (including the Cell Bank, Plant Cell Bank, DNA Bank, and BioInfo Bank), which is similar to the ATCC in the United States. The RIKEN Institutes are being remodeled, and a Center for Bioresources for Japan is being constructed on the Tsukuba site—similar to Jackson Laboratory in the United States as a resource for supplying mice for Japanese and other investigators

RIKEN is oriented towards pure science. It is reluctant to allow venture capital spin-off companies to be formed by employees. Also the system seems to be oriented along disciplinary lines (silos), and cross-disciplinary research is difficult. RIKEN does have a technology licensing office in the Wako site. Approval for clinical trials was done within the hospital at Tsukuba. We also toured a P-4 laboratory facility at the site. It was not currently being used.

R&D ACTIVITIES

Dr. Ohno received a large government grant from the STA for tissue engineering. It is approximately one million dollars per year and supports 10 principal scientists (exclusive of salaries). It had three more years to go. Dr. Ohno's research on lymph node tissue engineering has grown out of his main area of research, which is the immunotherapy of tumors. He has developed culture methods for the concentration and expansion of autologous cytotoxic T lymphocytes (CTLs) and natural killer cells (NK) from human patients. These can then be administered back to the patient with the hope that the patients' own immune system, if stimulated, can kill the tumor cells. That is the underlying hypothesis if tumor cells produce antigen to which CTL or NK cells can be activated with co-culture.

The CTL therapy has been tried in 10 patients with glioblastoma brain cancer who were no longer responsive to radiation or chemotherapy after surgery. Patient blood mononuclear cells are cultured with tumor cells or minced tumor tissue in a culture medium developed at RIKEN. After expansion and appropriate testing, these CTLs or NK cells are then injected into the patients. Most patients had some positive responses (9 out of 10) in terms of tumor regression, but tumors reoccurred in all patients.

Adverse reactions were generally just fever and minor bleeding problems. Less work has been done with NK cell preparations. The long-term goal is to generate NK cells and CTLs *in vivo* using a cell-based lymphoid production system rather than using *in vitro* culture systems. Work in developing this tissue-engineered system is just beginning, however.

The clinical work described above was done after *in vitro* evaluation of CTL or NK cell ability to kill tumor cells, but no animal work was performed.

Biomolecules

RIKEN has developed media for preferential expansion of CTLs or NK cells from peripheral blood mononuclear cells, employing cytokine cocktails.

Cells

The Gene Bank is a center for cell line repository, equivalent to ATCC. There is a catalogue and Web site (www.rtc.riken.go.jp) for researchers around the world. Culture of activated CTLs and NK cells is under investigation for cancer treatment.

REGULATORY ISSUES

Direct clinical trials are from *in vitro* work. The patent policy and support within RIKEN is interesting. It has been hard to get RIKEN to approve release of technology for private company development.

REFERENCES

RIKEN. 1999. Animal cells, plant cells, DNA clones, libraries, and bioinformatics. *General Catalog*. April. (Aalso available online at http://www.rtc.riken.go.jp.)

_____2000. *Riken*. Brochure.

_____2000. *Riken Gene Bank Newsletter*. No. 12, April.

_____1999. *Riken Gene Bank Newsletter*. No. 11, November.

_____1999. *Tsukuba Life Science Center*. Brochure.

Site: **Tokyo Institute of Technology**
4259 Nagatsuta-Cho
Midori-Ku Yokohama-Shi 226-8501
Tokyo, Japan
http://www.bio.titech.ac.jp

Date visited: 24 August 2000

WTEC Panelists: M. Mrksich (report author), H. Greisler, G. Holdridge, L. McIntire, N. Parenteau

Hosts: Professor Toshihiro Akaike, Department of Biomolecular Engineering, and Director, Research Center for Experimental Biology
Tel.: +81-45-924-5790; Fax: 81-45-924-5815; Email: Takaike@bio.titech.ac.jp

SUMMARY

The School of Bioscience and Biotechnology at the Tokyo Institute of Technology was established in 1990 to educate and train students in emerging biotechnology fields and was expanded two years later to include research teams in several thematic areas. Professor Akaike is head of the Biomaterial Design Group. His group combines a very strong position in polymer chemistry with cell biology to develop biomimetic approaches towards producing a bioartificial liver. The annual research budget for the effort is approximately $500,000, provided by MITI and the Ministry of Education.

SUMMARIES OF TECHNICAL PROJECTS

Professor Akaike is developing polymer scaffolds to serve as biomimetic matrices for the culture of liver cells. The approach emphasizes the role of carbohydrate ligands in mediating cell adhesion and regulating cell function. The Akaike group leverages its strong position in polymer chemistry to prepare artificial glycoconjugate polymers. These researchers have developed many synthetic approaches to access copolymers of polystyrene with carbohydrates.

The laboratory has developed a poly-N-*p*-vinylbenzyl-O-β-D-galactopyranosyl-[1,4]-D-gluconamide (PVLA) as a matrix for hepatocyte culture. The carbohydrate residues of the polymer interact with the asialoglycoprotein receptor (ASGP-R) of hepatocytes. The behavior of cells depends strongly on the coating density of the polymer. Low coating densities result in cells that are highly proliferative, while higher coating densities promote greater cell spreading and higher expression levels of ASGP-R. The researchers believe that this ability to control cell morphology and function will be important in the development of a bioartificial liver.

Other work has investigated apoptosis in mouse primary hepatocytes. The researchers have found that short peptides (Z-VAD-Fmoc and Z-LEVD-Fmoc) can prevent the INF-γ initiated apoptosis by blocking specific caspase proteases. They are applying these findings to the development of nanoparticles for delivery of the inhibitory peptides to cells. This work is using 100 nm PLA/PVLA particles that are loaded (non-covalently) with the peptides.

SUMMARY

This group has a strong position in polymer chemistry and has developed general routes towards synthetic glycopolymers. Their work with hepatocyte culture demonstrates clearly the value in controlling the structure and properties of synthetic matrices for culturing cells. A closer collaboration with a sophisticated cell biology group could lead to novel technologies that have high impact. The patenting and commercialization of this work remains undeveloped. While the Institute is now making this issue a priority, there are still no clear paths by which investigators can obtain advice and funding for submitting patent applications.

REFERENCES

Tokyo Institute of Technology. n.d. Tokyo institute of technology graduate school of bioscience and biotechnology. Catalog.

Ajioka I., T. Akaike, and Y. Watanabe. 1999. Expression of vascular endothelial growth factor promotes colonization, vascularization, and growth of transplanted hepatic tissues in the mouse. *Hepatology* 29 (2):396-402.

Akaike, T., M. Goto, A. Kobayashi, K. Kobayashi, and S. Tobe. 1993. Design of hepatocyte-specific extracellular matrices for hybrid artificial liver. *Gastroenterologica Japonica* 45-52.

Ferdous, A., T. Akaike, and A. Maryuma. 2000. Inhibition of sequence specific protein-DNA interaction and restriction endonuclease cleavage via triplex stabilization by poly(L-lysine)- graft- dextran copolymer. *Biomacromolecules* 1 (2):186-193.

Goto, M., T. Akaike, C-W Chang, A. Kobayashi, K. Kobayashi, S. Kojima, A. Maeda, T. Shinoda, and H. Yura. 1994. Lactose-carrying polystrene as a drug carrier: investigation of body distributions to parenchymal liver cells using I-labeled lactose-carrying polystrene. *Journal of Controlled Release* 28:223-233.

Ise, H., T. Akaike, A. Ferdous, M. Nagakoa, and S. Takashima. 1999. Analysis of cell visibility and differential activity of mouse hepatocytes under 3d and 2d culture in agarose gel. *Biotechnology Letters* 21:209-213.

Kim, S-H, T. Akaike, C-S Cho, and M. Goto. 2000. Specific adhesion of primary hepatocytes to a novel glucose-carrying polymer. *Biotechnology Letters* 22:1049-1057.

Maruyama, A., T. Akaike, T. Ishihara, and M. Katoh. 1997. Comb-type polycations effectively stabilize DNA triplex. *Bioconjugate Chemistry* 8 (1):3-6.

Mikawa, M., T. Akaike, M. Brautigam, A. Maruyama, and N. Miwa. 2000. Gd3+ -loaded polyion complex for pH depiction with magnetic resonance imaging. 390-395.

Ohgawara, H., T. Akaike, Q. Fu, S. Karibe, M. Kawamura, A. Kobayashi, and Y. Omori. 1994. Development of a method for embedded-culture of pig pancreatic islet-like cell clusters in agarose containing maltose-carrying polystyrene (hevm) and nicotinamide. *Cell Transplantation* 3 (1):83-89.

Sasagawa, T., T. Akaike, and M. Hlaing. 2000. Synergistic induction of apoptosis in murine hepatoma hepa 1-6 cells by ifn~y and tnf-a. *Biochemical and Biophysical Research Communications* 272:674-680.

Takei, R., T. Akaike, Y. Ebara, H. Ise, A. Maruyama, Y. Okahata, and K-H Park. 1997. Coated antireceptor antibody as an extracellular matrix for liver tissue engineering. *Tissue Engineering* 3 (3):281-288.

Site: **Tokyo Women's Medical University**
Institute of Biomedical Engineering
8-1 Kawada-cho, Shinjuku-ku
Tokyo, 162-8666, Japan
http://www.twmu.ac.jp/

Date visited: 26 August 2000

WTEC Attendees: M. Mrksich (report author), H. Greisler, G. Holdridge, L. McIntire, N. Parenteau

Hosts: Professor T. Okano (not present), Director
Dr. Masayuki Yamato, Research Assistant Professor
Tel: +81- 3-3353-8111, x30232; Fax: + 81- 3-3559-6046;
Email: myamato@lab.twmu.ac.jp
Dr. Yoshihiro Muragaki, MD, PhD, Department of Surgery, Neurological Institute
Email: ymuragaki@nij.twmu.ac.jp
Dr. Tatsuya Shimizu, MD, Assistant Professor; Email: tshimizu@lab.twmu.ac.jp
Madoka Sugiura, Hitachi Ltd., Marketing Dept., Medical Systems Div.
New Marunouchi Bldg., 5-1 Marunouchi 1-chome
Chiyoda-ku, Tokyo, 100-8220 Japan; Tel.: +81-3-3212-1111;
Fax: +81-3-3212-367; Email: m-sugiura@med.hitachi.co.jp

SUMMARY

The Institute for Biomedical Engineering at Tokyo Women's Medical University is directed by Professor T. Okano. His group includes an associate professor (M. Iijima), four research associate professors, five postdoctoral fellows, two lecturers, and several technical staff. Professor Okano's background is in polymer chemistry, and the institute's programs derive from a leading position in functional polymer materials. The position in fundamental biology, by comparison, is not as strong. The institute is exceptionally well funded, with an annual budget of approximately ¥3 billion. Included in this funding is ¥1.6 billion provided by MITI for the development of medical technologies that are near commercialization. The institute is notable for the sophisticated approach to protecting intellectual property and planning for commercialization.

SUMMARIES OF TECHNICAL PROJECTS

Much of the work in the Institute for Biomedical Engineering is based on a class of polymer films that are thermally responsive and undergo a phase transition near 32º C to switch between a swollen and condensed state. Because the former is inert to protein adsorption and cell adhesion, while the latter is a good substrate for cell adhesion, these polymer films can release adherent cells when they are cooled below the transition temperature. The institute has exploited this dynamic property in several projects. Summaries follow.

Cell Sheet Engineering

The institute has developed a method based on electron beam grafting for modifying Falcon tissue culture dishes with the poly(N-isopropylacrylamide) thermally responsive polymer. Cells attach and proliferate on these substrates at 37º C, but on removal from an incubator, the substrates cool to room temperate and efficiently release the adherent cells. When endothelial cells are grown to a confluent state, they can be released from the substrate to give a nondissociated cell sheet. Significantly, these sheets are associated with ECM and therefore can be transferred to other substrates to which they attach efficiently. Institute researchers have cultured keratinocytes to serve as artificial epidermis and have evaluated the sheets for regeneration of wound sites in nude mice. The significant advance with these methods is that they avoid the need to use proteases to harvest the sheet, and hence they reduce pathological activation and infection of the tissue. The cell sheets have been successfully transferred to polyvinyldifluoride (PVDF) hydrophilic membranes, which

provide mechanical strength to the sheets. Importantly, the cell sheets remain viable and are stable to the processing steps.

Institute researchers have employed photolithographic techniques to pattern the thermally responsive polymer to substrates. The resulting substrates enable new strategies for patterning cellular co-cultures. In one example, hepatocytes were allowed to attach and grow to a confluent layer; lowering of the temperature below 32º C resulted in the release of hepatocytes from patterned regions of the substrate, which could then be seeded with a second cell type (in this work, endothelial cells). The institute is pursuing this work to develop new routes towards artificial kidney and liver.

Cardiac Tissue Engineering

Dr. Tatsuya Shimizu described a program to culture heart tissue from pig cells for the treatment of heart failure. Cardiomyocytes were cultured on the thermally responsive substrate and then released and transferred to PVDF membranes to prevent subsequent shrinkage. A cell sheet prepared in this way could be transferred to a second confluent layer of cells to form a double cell layer that formed junctions between the two sheets. Current work in the Institute is developing methods for the three-dimensional culture of cells and is pursuing development of these cultures for implantation.

Computer-Aided Surgery

Dr. Yoshihiro Muragaki described a large program (¥2 billion annual) in collaboration with Hitachi and Toshiba Corporations to develop a real-time imaging system to support the surgical removal of brain tumors. This program is technically very sophisticated and is at the point of commercialization. Briefly, the procedure starts by using functional electrode arrays to map motor functions in the brain, so as to identify volume elements that should be protected from surgical intervention. This information is provided to a three-dimensional map of the brain, which is used to guide the microsurgery. Because the operation alters the form of the brain, it is necessary to image the brain several times during the surgery, and to continuously update the active map. This technology should provide for the removal of 95% of tumor mass with minimal consequence to normal tissue. The open MRI system in use for this project is unique.

SUMMARY

The Institute for Biomedical Engineering has been a leader in the development of functional polymer substrates for cell culture. Its work over the past year has applied these materials advances to a number of tissue-engineering applications. Were they to build a strong position in the fundamental biology, these researchers would be leaders in developing a new generation of tailored biomaterials. The institute is also among the most sophisticated in Japan in pursuing protection of valuable intellectual property and in planning for commercialization of key technical developments.

REFERENCE

Tokyo Women’s Medical University. 2000. ME Cooperation Laboratory System. Brochure.

Site: **University of Tokyo**
Department of Biomedical Engineering
Postgraduate School of Medicine
7-3-1 Hongo, Bunkyo-ku
Tokyo, 113-0033, Japan

Date visited: 21 August 2000

WTEC Attendees: H.P. Greisler (report author), G. Holdridge, L. McIntire, N. Parenteau

Hosts: Joji Ando, MD, PhD; Professor of Biomedical Engineering
Tel: 81- 3-5841-3659, Fax: 81-3-5800-6928; Email: joji@m.u-tokyo.ac.jp

INTRODUCTION

Professor Ando has an active basic research laboratory in the University of Tokyo's Department of Biomedical Engineering and in addition is a practicing cardiologist who devotes one day per week to clinical activities. His research program focuses on cellular biomechanics, cellular responses to hemodynamic forces. While this work is directly applicable to tissue engineering, his lab does not focus on such applications.

The laboratory's funding is primarily from the Ministry of Education, Culture, and Sports with additional funds from the Science and Technology Agency. This particular laboratory receives no industry support. Professor Ando's research budget is ¥50,000,000 per year, not including staff salaries, and is subject to competitive renewals as frequent as every second year.

Major equipment items can be procured either from grant funds or by successfully competing for separate equipment support from the University of Tokyo.

There are three professors at the Department of Biomedical Engineering, Dr. Ando among them. The other two focus their research on the bioartificial heart and on biomagnetism. Dr. Ando's group includes three staff members below the level of professor and three or four students, both PhD and MD, who generally spend four years in the lab.

R&D ACTIVITIES

Cells

The research focus is on the response of HUVECs to mechanical forces. Other sources of primary human endothelial cells, (e.g., microvascular or saphenous vein) could be available through the hospital but are not used.

The primary models for studying shear forces include the parallel plate and the cone and plate systems. Cycle strain is studied using a pulsatile perfusion apparatus with cells on a 40 mm x 4 mm silicone tube.

Using the parallel plates shear stress system, Dr. Ando showed laminar shear to stimulate nitric oxide (NO) production assayed by intracellular cGMP. He showed shear to down-regulate VCAM-1 mRNA. Using deletion analysis by which the gene is made progressively shorter and transfecting the HUVECs with the truncated VCAM-1 gene, the shear stress responsive element (SSRE) was found to be between -0.7 and -0.3kB, an area with 2 AP-1 binding sites.

Post-transcriptional regulation of genes in response to shear also occurs. For example, shear induces an up-regulation of GM-CSF mRNA in HUVECs, which was found to be due to a change in mRNA stabilization. Species differences likely exist based on reported literature comparing HUVECs to murine ECs.

Dr. Ando's group has been using mRNA differential display techniques to identify shear stress responsive genes. Assaying 1507 genes, 60 were differentially expressed, 33 up-regulated and 27 down-regulated. Assuming the entire human genome to contain approximately 100,000 genes, this same 4% incidence of differential expression would suggest 400 genes to be responsive to shear stress. Sixteen of the shear stress responsive genes were cloned and sequenced. Homology searching revealed 6 known and 10 unknown genes. Known genes included those encoding laminin B1 chain, H^+-ATP synthase coupling factor 6, lysyl oxidase, myosin light chain kinase, interleukin-8 receptor, and NADH dehydrogenase.

Signal transduction mechanisms are actively under investigation. Calcium influx into HUVECs on coverslips subjected to a stepwise increase in flow is visualized by a fluorescent Ca^{++} indicator, Fura-2/AM (in the presence of ATP). This correlates with an increase in P2X receptor subtype 4 and is blocked by transfection of HUVECs with antisense P2X4. This calcium influx begins at one edge of the cell and moves progressively across the cell, seen by high-speed fluorescence imaging. The leading edge is rich in caveolae, suggesting that caveolae may be the sites at which the flow signal enters and the calcium response begins. This is also the site of an IP_3 receptor-like protein, the plasmalemmal Ca^{++} pump, and of eNOS co-localization.

Biomolecules

This lab does not directly focus on biomolecules as related to tissue engineering *per se*. However, the basic research on shear stress responsive genes may ultimately provide clues relevant to cellular preconditioning for specific tissue-engineering applications such as vascular tissue engineering. (See details under "Cells" section above).

Engineering Design Aspects

The Ando group utilizes cell monolayer cultures with *in vitro* perfusion in parallel plate and cone-plate systems and performs cyclic strain studies using cultures on silicone tubes (40 mm x 4 mm).

LEGAL AND REGULATORY ISSUES

Although Dr. Ando's research is not funded by industry, he is permitted to apply for such funds. The rights to the results of such research would then be determined on a case-by-case basis through negotiations between the University of Tokyo and the industry partner.

REFERENCES

Ando, J., R. Korenaga, A. Kamiya. 2000. Shear stress-dependent regulation of endothelial cell functions. In *New Frontier in Vascular Biology: Thrombosis and Hemostasis.* Eibun Press, Ltd.

Ando, J., H. Tsuboi, R. Korenaga, K. Takahashi, K. Kosaki, M. Isshiki, R. Tojo, Y. Takada, and A. Kamiya. 1996. Differential display and cloning of shear stress response messenger RNAs in human endothelial cells. *Biochemical and Biophysicial Research Communications* 225:347-351.

Isshiki, M., J. Ando, R. Korenaga, H. Kogo, T. Fujimoto, T. Fujita, and A. Kamiya. 1998. Endothelial Ca^{2+} waves preferentially originate at specific loci in caveolin-rich cell edges. *Proc. Natl. Acad. Sci.* 95:5009-5014.

Korenaga, R., J. Ando, K. Kosaki, M. Isshiki, Y. Takada, and A. Kamiya. 1997. Negative transcriptional regulation of the VCAM-1 gene by fluid shear stress in murine endothelial cells. *Am. J. Physiol.* 273:C1506-C1515.

Kosaki, K., J. Ando, R. Korenaga, T. Kurokawa, and A. Kamiya. 1998. Fluid shear stress increases the production of granulocyte-macrophage colony-stimulating factor by endothelial cells via mRNA stabilization. *Circulation Research* 82:794-802.

Takada, Y., C. Kato, S. Konso, R. Korenaga, and J. Ando. 1997. Cloning of cDNAs encoding g protein-coupled receptor expressed in human endothelial cells exposed to fluid shear stress. *Biochemical and Biophysicial Research Communications* 240:737-741.

Yamamoto, K., R. Korenaga, A. Kamiya, and J. Ando. 2000. Fluid shear stress activates Ca2+ influx into human endothelial cells via P2X4 purinoceptors. *Circ. Res.* 87:385-391.

Yamamoto, K., R. Korenaga, A. Kamiya, Z. Qi, M. Sokabe, and J. Ando. 2000. P2X4 receptors mediate ATP-induced calcium influx in human vascular endothelial cells. *Am. J. Physiol. Heart Circ. Physiol*. 279:H285-H292.

Site: **University of Tsukuba**
1-1-1, Tennoudai
Tsukuba, 305-0006, Japan

Date visited: 23 August 2000

WTEC Attendees: H.P. Greisler (report author), G. Holdridge, L. McIntire, M. Mrksich, N. Parenteau

Hosts: Professor Norio Ohshima, Dean, Master's Programs
Tel.: +81-298-53-3084; Fax: +81-298-53-7379;
Email:ohshima@md.tsukuba.ac.jp
Dr. Yoichi Iwasaki, Vice President for Research and Development
Email: iwasaki@rccp.tsukuba.ac.jp
Professor Masayuki Yamamoto
Professor Katsutoshi Goto, Professor of Pharmacology; Director, Tsukuba Advanced Research Alliance Center (TARA)
Dr. Hirotoshi Miyoshi, Assistant Professor

INTRODUCTION

The University of Tsukuba traces its history to 1872, but 1973 was the foundation date of the current University of Tsukuba as a new national university. The stated aim of the university is to establish a free, deep, and close exchange of basic and applied sciences with educational and research organizations and academic communities in Japan and overseas. The university has 14,077 students, including 2,253 in master's degree programs and 2,391 in doctoral degree programs. Research is organized in 26 research institutes, 3 special research projects, and 21 research and educational centers. Total expenditures were ¥68.19 billion. The university has had 2 Nobel laureates.

The University of Tsukuba is located within Tsukuba Science City, which has 50 national centers and approximately 300 industrial research facilities. The total research budget combined is ~¥3 trillion per year.

The organizational and managerial systems of this university are detailed in the handout given to the WTEC team entitled, "Outline of the University of Tsukuba 1999-2000." Among the 26 research institutions within the university are the following:

- Institute of Basic Medical Sciences (17 Professors, 64 staff)
- Institute of Clinical Medicine (26 Professors, 210 staff)
- Institute of Community Medicine (7 professors 23 staff)
- Institute of Applied Biochemistry (17 professors, 50 staff)
- Institute of Biological Sciences (18 professors, 57 staff)
- Institute of Chemistry (10 professors, 36 staff)
- Institute of Engineering Mechanics and Systems (26 professors, 67 staff)
- Institute of Information Science and Electronics (23 professors, 65 staff)

Among the 21 university research and eductional centers is TARA, the Center for Tsukuba Advanced Research Alliances, one of the objects of the WTEC team's visit. The objectives of TARA are (1) the creation of basic Japanese research and the creation of novel fields in advanced interdisciplinary science by the collaboration of industry, the government and universities, and (2) the application of research results to society, thus providing new demands for venture business.

Each research aspect within TARA is reevaluated every 7 years. Similarly, a 7-year tenure system was introduced for the professors and assistant professors, who are also reevaluated on the same schedule.

The review of research projects (every 3 years) and of research aspects (every 7 years) incorporates the following 5 principles: (1) alliance with other institutions, (2) competition (3) evaluation (4) priorities, and (5) social contribution.

TARA is organized as 7 projects, each headed by a professor: (1) molecular and developmental biology, (2) regulation of biological function, (3) nanostructures and basic materials, (4) supermaterials/instrumentology, (5) information management, (6) human beings in the ecosystem, and (7) intellectual property and technology transfer.

The TARA building, completed in 1998, was designed to maximize flexibility and interaction. It includes very well equipped core facilities, including pathology and murine embryonic stem cell facilities.

Another highlight of the WTEC team's visit, the project "Bio-Process Engineering of Functional Regeneration of Cultured Animal Cells," is led by Professor Norio Oshima, Professor, Department of Biomedical Engineering, Institute of Basic Medical Sciences. The overall project can be subdivided into 3 units: (1) Functional Regeneration of Cultured Hepatocytes, (2) Functional Regeneration of Chondrocytes and Bone Marrow Cells, and (3) Functional Regeneration of Neovasculature and Blood Vessels. The overall budget for this project is $1,000,000 per year; the Ministry of Education provides 20% of this as part of JSPS (Japan Society for the Promotion of Science). This 5-year grant with the opportunity for competitive renewal includes funding for 4 post-docoral fellows.

R&D ACTIVITIES

Biomaterials

The primary scaffold biomaterial used for a variety of tissue-engineering applications is polyvinyl formal resin (PVF). The highly porous and reticulated PVF is synthesized by reacting polyvinyl alcohol with formaldehyde. The three-dimensional reticulated structure with continuous interconnecting pores is formed by extracting the presoaked pore-forming agent. The resin has a high porosity of approximately 90%, with pore size, which can be controlled, ranging from 5-1000 μm. For most applications the group uses port sizes of 100-200 μm.

In addition, the group is utilizing scaffolds of polylactic capralactone for chondrocyte studies.

In typical tissue-engineering applications, any of several cell types (see "Cells" section below) is seeded into the PVF or PCL resins in the presence of cytokines and placed into a packed bed bioreactor (see "Engineering Design Aspects" section below).

Cells

Current studies in the Oshima lab utilize hepatocytes, chondrocytes derived from mesenchymal stem cells, and stromal cells derived from mesenchymal stem cells.

The clinical goal for the hepatocyte studies is for a 2-3 week period of support for patients with acute hepatic failure, after which hepatic regeneration may occur. This could also be viewed as a bridge to transplant, although that is not the stated goal. Much of this project is under the direction of Dr. Hirotoshi Miyoshi, assistant professor in the Department of Biomedical Engineering. Thus far, studies have focused on *in vitro* profusion of rat hepatocytes and are currently being extended to porcine hepatocytes, which will ultimately be selected for clinical application. Following 1-2 week *in vitro* perfusion of the rat cells, 30% of metabolic activity is retained, as measured by albumin secretion. In a 1998 publication in the *Journal of Biomaterial Science Polymer Edition*, the group reported better retention of albumin secretion by cells immobilized in PVF resin vs. in monolayer culture without PVF, both in static conditions (Miyoshi, Ookawa, and Oshima 1998). A publication by the group in 2000 in the *ASAIO Journal* reported the use of fetal liver cells, which show better proliferative activity *in vitro* compared to mature hepatocytes (Miyoshi et al. 2000). Optimal conditions utilized aMEM media for the initial 10 days to encourage cell growth, followed by WE (Williams E) media to enhance albumin secretion. The high PVF porosity allowed a cell density of 1×10^7 cells/cm^3

PVF in a packed bed reactor loaded with cubic PVF resins and retention of metabolic activity for over 20 days, whereas that in conventional monolayer cultures rapidly decreased.

Related recent work has focused on the clonal expansion of murine multipotent stem-cell-like progenitor cells. As reported in the *Daily Yomiuri*, August 19, 2000, these cells were propagated, transfected with a marker GFP gene, and transplanted into mouse livers by this group, led by Hideki Taniguchi and supervised by Professor Takashi Fukao. Forty days later, after only 100,000 stem cells (0.5% of the cells in a complete mouse liver) were transplanted, 20-40% of the liver cells were GFP-positive and were capable of albumin secretion.

Bone-marrow-derived mesenchymal stem cells are used for generation of chondrocytes. The stem cells first differentiate on perfused PVF of PLC sponges under pressure. Preliminary work is in progress transplanting these cells into the subcutaneous tissue of nude mice.

Stromal cells are also derived from murine bone marrow mesenchymal stem cells. PVF enhances cell survival, the best conditions using Type 1 collagen coating and a small 130 μm pore size. By FACS analyses >90% of cells are macrophage with additional mononuclear cells, granulocytes, and erythrocytes. Cells retained viability up to 6 weeks. *In vivo* transplantation experiments into irradiated mice (subcutaneous) revealed 15% survival of transplanted cells (Tun et al. 2000). Currently, *in vitro* conditions utilize a mixture of IL-3, IL-6, erythropoietin, and hematopoietic growth factors (HGFs). The group hopes to next define specific cytokine environments to induce specific cell types including T-lymphocytes, B-lymphocytes, etc.

Biomolecules

In vitro culture of various cell types on PVF resins utilizes coating of resins with cell adhesive proteins. Fetal hepatocytes cultured on laminin retain better albumin secretion, and there is a suggestion of improvement using Type 4 collagen. Stromal cells are cultured on Type 1 collagen.

As described above, hematopoietic cells are provided a mix of IL-3, IL-6, HGF, and erythropoietin. The group plans studies to identify specific cytokine regimes to optimize the generation of specific hematopoietic cells, including T-lymphocytes and B-lymphocytes.

Professor Katsutoshi Goto of the Department of Pharmacology is a collaborator and focuses primarily on endothelin, which was originally described by him in 1987. In recent studies, ET-1 was found to promote proliferation of cultured astrocytes, blocked by a monoclonal antibody to ET-1.

Engineering Design Aspects

For tissue-engineering applications the group uses primarily a packed-bed-type bioreactor constructed by loading PVF resins into a cylindrical column. Columns described in *Artificial Organs* (Oshima, Yanagi, and Miyoshi1997) were 12 mm inside diameter x 45 mm height and 20 mm inside diameter x 55 mm height. Larger bioreactors are now also in use. As detailed in *ASAIO Journal* (Yanagi, Miuoshi, and Oshima 1998), culture medium (50 ml) is perfused at 17 ml/minute into the bottom port through an oxygenator introducing a mixture of 5% CO_2, O_2 and N_2. Dissolved oxygen concentration is controlled by adjusting gas-mixing ratios.

Another major focus of the lab is on microcirculation studies. Using confocal microscopy and intravital dyes (FITC, rhodamine) real-time *in vivo* visualization of leukocyte adherence within tumor microvessels is observed.

Studies are also underway on the effect of PTCA balloon inflation on endothelial cell adherence to glass tubes. Under pressures used in clinical PTCA, all cells desquamate. Reduction of the rate of balloon deflation enhanced cell retention. The group plans to develop conduits more arterial-like then glass to pursue these studies.

LEGAL AND REGULATORY ISSUES

Because the University of Tsukuba is a government institution, patents derived from the work would be owned by the government, and income derived from commercialization would be divided between the government and the university. It was described as difficult for a scientist to pursue a patent individually, although industry-supported work would yield industry-owned patents.

REFERENCES

University of Tsukuba. Handout on Master's Program in Medical Sciences. .

———. An International Visitor's Guide to the University of Tsukuba.

Bibliography of selected articles published by the University of Tsukuba:

Daily Yomiuri. August 19, 2000. Research team cultivates liver tissue from stem cells.

Fan, J., M. Araki, L. Wu, M. Challah, H. Shimoyamada, R. Lawn, H. Katuta, H. Shikama, and T. Watanabe. 1999. Assembly of lipoprotein (a) in transgenic rabbits expressing human apolipoprotein (a). *Biochemical and Biophysicial Research Communications* 255:639-644.

Goto, K. 1999. History of endothelin. In R.G. Goldie and D.W.P. Hay, eds. *Pulmonary Actions of the Endothelins.* Basel/Switzerland: Birkhuser Verlag.

Miyoshi, H., K. Ookawa, and N. Oshima. 1998. Hepatocyte culture utilizing porous polyvinyl formal resin maintains long-term stable albumin secretion activity. *J. Biomater. Sci.* Polymer Ed. 9:227-237.

Miyoshi, H., T. Ehashi, H. Ema, H.C. Hsu, H. Nakauchi, and N. Oshima. 2000. Long-term culture of fetal liver cells using a three-dimensional porous polymer substrate. *ASAIO Journal* 397-402.

Oshima, N., 1998. Bioprocess engineering of functional regeneration of cultured animal cells. In Y. Ikada and S. Enomoto, eds. *Tissue Engineering for Therapeutic Use 2,* pages 113-118. . Elsevier Science..

Oshima, N., K. Yanagi, and H. Miyoshi. 1997. Packed-bed type reactor to attain high density culture of hepatocytes for use as bioartificial liver. *Artificial Organs* 21:1169-1176.

Suzuki T., K. Yanagi, K. Ookawa, K. Hatakeyama, and N. Oshima. 1998. Blood flow and leukocyte adhesiveness are reduced in the microcirculation of a periotoneal disseminated colon carcinoma. *Annals of Biomedical Engineering* 26:803-811.

Tun, T., H. Hiyoshi, H. Ema, H. Nakauchi, and N. Oshima. 2000. New type of matrix support for bone marrow cell cultures: in vitro culture and in vivo transplantation experiments. *ASAIO Journal* 1-5.

Yanagi, K., H. Miuoshi, and N. Oshima. 1998. Improvement of metabolic performance of hepatocytes cultured in vitro in a packed-bed reactor for use as a bioartificial liver. *ASAIO J.* 44; M436–M440.

APPENDIX D. SUMMARY OF THE JUNE 2000 U.S. REVIEW WORKSHOP

INTRODUCTION

The National Science Foundation and other agencies of the U.S. government have asked the World Technology (WTEC) Division of the International Technology Research Institute to perform an assessment of status and trends in tissue-engineering research and applications around the world in comparison to that in the United States. The purpose of this study is to assess the U.S. tissue-engineering R&D effort in comparison to activities abroad; provide the scientific/engineering community with a critical view of the field and identify the most promising areas for future research and industrial development; stimulate the development of an inter-disciplinary and international community of tissue engineering researchers; and identify opportunities for international collaboration in the field. WTEC has recruited a panel of U.S. experts in the various related fields to perform this assessment (see inside cover). The panel is charged with analyzing and comparing research in the United States with that being pursued abroad. This panel visited relevant R&D facilities in Japan and Western Europe during the summer of 2000. Prior to these visits the panel first needed to develop an understanding of the state of the art in these technologies in the United States.

Towards this end, WTEC invited leading U.S. tissue engineering researchers to a workshop held at NIH in Bethesda, MD, on June 5 and 6, 2000. A volume of collected papers presented at the workshop is available on the Internet at http://itri.loyola.edu/te/usws/welcome.htm. Paper authors were asked to provide a broad description of all related U.S. work in their respective fields (i.e., not necessarily just the activities in their own laboratories). Authors were chosen to be representative of cutting-edge U.S. research in each of the topic areas. The following excerpts from the full report are the overviews of each session prepared by the respective WTEC panelists.

BIOMATERIALS (LINDA GRIFFITH)

Biomaterials are a critical enabling technology for virtually every tissue-engineering application. The most prominent role biomaterials play is creation of three-dimensional scaffolds to either guide tissue growth into acellular implants or to provide structure for organizing dissociated donor cells into appropriate tissue structures either *in vitro* or *in vivo*. In this context, both bulk (e.g., strength, degradability) and surface (or cell-interacting) properties of the material are important. The material properties also determine what types of device micro- and macro-architectures can be achieved. Among materials currently used in tissue engineering, bulk and surface properties are generally intertwined. Often, materials are chosen for their favorable bulk properties and their relative lack of unfavorable biological interactions, such as immunogenicity. Biomaterials are also increasingly playing a role as delivery devices for proteins (e.g., growth factors) or genes that affect the tissue regeneration process and in many cases may serve a dual role as scaffold and as delivery device.

Biomaterials used in tissue engineering can be categorized according to several schemes: material source, degradation properties, and general mechanical properties. With the exception of ceramic and other inorganic materials used in some hard tissue applications, biomaterials used in tissue engineering are organic polymers. Polymers may be derived from natural sources of animal or plant origin (e.g., Type I collagen or alginate); they may be synthesized from defined organic monomers (e.g., degradable polyesters); or created as semisynthetic hybrids by adding biomolecules to synthetic polymers or synthetic peptides or polymers to biomolecules (e.g., attaching RGD adhesion peptides to fibrin or hyaluronic acid). All three sources are being pursued in both commercial development and academic research. Regardless of source, most tissue-engineering applications require degradable or resorbable polymers. Devices made from permanent materials are viewed as a clinical stepping stone to degradable devices. Finally, mechanical properties of tissue-engineering polymers may broadly be divided into gels (e.g., alginate, PEO, collagen) and water-insoluble materials (e.g., polylactic acid, cross-linked collagen). Gels are typically preferred when *in situ* formation of a device is desired.

Several key themes in biomaterials research and development are emerging as knowledge is gained from animal and human studies across a variety of applications. First, existing materials that are already clinically accepted, such as collagen and polylactide-co-glycolide, are often adequate for first-generation products and may even be optimal for later-generation products when economics are considered. However, new materials with improved properties designed specifically for tissue engineering are needed. The perception that the field would not embrace development of new materials due to potential liabilities and FDA regulations has faded. The tissue engineering community is on the whole highly positive about the effort of the FDA to develop appropriate guidelines for testing new materials and devices, given the specific demands of tissue engineering and the degree to which the FDA has reached out to the tissue engineering community for input.

A substantial limitation of synthetic materials is often inadequate interactions with cells. Although such shortcomings may be addressed by changing the general physicochemical properties of the surface, the more substantial trend is toward endowing synthetic materials with molecular entities that interact with cell surface receptors. Among the major challenges in this regard are understanding how cells respond to such signals quantitatively, in other words, developing the appropriate design principles for new materials. For example, cell migration into a device depends not just on whether a ligand is there or not, but depends in a nonlinear fashion on how much ligand is there. Thus, the development of biomaterials is iterative: new materials feed into studies of basic cell biology to provide new design principles. A second major challenge is keeping synthesis steps to a minimum to ensure that materials can be made reproducibly and economically. Finally, methods for processing materials into specific microscopic and macroscopic architectures suitable for organizing 3D tissue growth are still fairly primitive. Most tissues are arranged in complex hierarchical structures, with function deriving from form. The roles scaffolds play in assisting the development of appropriate tissue architecture is still poorly understood in most cases. New methods are emerging, however, from the field of rapid prototyping, providing both research tools and the means to translate research findings into GMP manufacturing.

The session was organized along the lines of materials categorized by source (ceramics, natural polymers, synthetic polymers, hybrids) with a final perspective on the cell-material interface.

References

Baldwin, S.P., and W.M. Saltzman. 1998. Materials for protein delivery in tissue engineering. *Advanced Drug Delivery Reviews* 33:71-86.

Bonadio, J., E. Smiley, P. Patil, and S. Goldstein. 1999. Localized, direct plasmid gene delivery *in vivo*: Prolonged therapy results in reproducible tissue regeneration. *Nat. Med.* 7:753-759.

Brocchini, S., K. James, V. Tangpasuthadol, and J. Kohn. 1998. Structure-property correlations in a combinatorial library of degradable biomaterials. *Journal of Biomedical Materials Research* 42:66-75.

Chamberlain, L.J., I.V. Yannas, H.P. Hsu, G.R. Strichartz, and M. Spector. 2000. Near-terminus axonal structure and function following rat sciatic nerve regeneration through a collagen-GAG matrix in a ten-millimeter gap. *J. NeuroSci Res.* 60:666-677.

Elisseeff, J., K. Anseth, D. Sims, W. McIntosh, M. Randolph, and R. Langer. 1999. Transdermal photopolymerization for minimally invasive implantation, *Proceedings of the National Academy of Sciences of the United States of America* 96:3104-3107.

Griffith, L.G. 2000. Polymeric Biomaterials. *Acta Mater.* 48:263-277.

Hubbell, J.A. 1999. Bioactive biomaterials. *Current Opinion in Biotechnology* 10:123-129.

———. 1998. Synthetic biodegradable polymers for tissue engineering and drug delivery. *Current Opinion in Solid State & Materials Science* 3:246-251.

Kim, B.S., A.J. Putnam, T.J. Kulik, and D.J. Mooney. 1998. Optimizing seeding and culture methods to engineer smooth muscle tissue on biodegradable polymer matrices. *Biotechnology and Bioengineering* 57:46-54.

Niklason, L.E., J. Gao, W.M. Abbott, K.K. Hirschi, S. Houser, R. Marini, and R. Langer. 1999. Functional arteries grown *in vitro*. *Science* 284:489-493.

Petka, W.A., J.L. Harden, K.P. McGrath, D. Wirtz, and D.A. Tirrell. 1998. Reversible hydrogels from self-assembling artificial proteins. *Science* 281:389-392.

Rowley, J.A., G. Madlambayan, and D.J. Mooney. 1999. Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials* 20:45-53.

Shi, H.Q., W.B. Tsai, M.D. Garrison, S. Ferrari, and B.D. Ratner. 1999. Template-imprinted nanostructured surfaces for protein recognition. *Nature* 398:593-597.

Yu, Y.C., V. Roontga, V.A. Daragan, K.H. Mayo, M. Tirrell, and G.B. Fields. 1999. Structure and dynamics of peptide-amphiphiles incorporating triple-helical protein-like molecular architecture. *Biochemistry* 38: 1659-1668.

CELLS (NANCY PARENTEAU)

Tissue engineering has one reactive component, cells. Their character, behavior and response to injury, repair, and biomaterials are all integral aspects of tissue engineering. Ultimately, cells will form the tissues of interest whether *in vivo* or outside the body. This section discusses the strategy of sourcing and using cells for tissue engineering and cellular therapy.

There are a number of sources for cells: autologous (from one's own body), allogeneic (from another human donor), or xenogeneic (sourced from animals). Once a source is identified, the next challenge is to propagate these cells and reintroduce them in the body in a meaningful way. As the engineering of biomaterials, biopolymers, and natural materials and systems improves, the limiting factor in tissue engineering will become the biology.

The notoriety of stem cell research has recently focused science and the public interest on this particular cell source as having potential for a wide variety of medical applications. Knowledge of cell regulation, cell plasticity, and control of differentiation is being gathered at a rapid pace. This must be combined with the multidisciplinary aspects of tissue engineering as a way to deliver the cells and promote a positive outcome: formation of new functional tissue. It is therefore important that stem cells be considered as an important element for tissue engineering.

A source for cells, a way to cultivate the cells, a way to direct proper differentiation of the cells to achieve function, and an understanding of the *in vivo* reaction and interaction are all key elements that will enable us to succeed in developing useful products or therapies from tissue engineering.

An autologous source for cells has been thought to be the most direct answer. This stems from a desire to avoid regulatory hurdles, enabling the therapeutic concept to be studied in humans earlier, and if promising, made available to patients earlier. It is clear from Dr. DuMoulin's discussion that safety considerations are still considerable when using autologous cells, particularly if one hopes to achieve commercial scale. Safety considerations include not only testing of reagents that will come in contact with the cells, but also testing and monitoring of procedures to ensure that each individual will receive functional material. Once beyond proof of concept, one of the constant challenges of processing autologous cells is achieving a consistent outcome given a starting material that will often vary in quality, amount, and behavior. The processing of autologous cells, even for a relatively simple procedure, requires extremely robust protocols. Documentation, monitoring outgoing product, and tracking patient outcome presents formidable, but necessary, hurdles to ensure effective, safe material to the patient and are a requirement in the United States for commercial enterprises. (Please refer to the Regulatory section for more information.)

Cell expansion is for a single patient; therefore limitations in expansion capabilities are less of an issue compared to an allogeneic source that must serve many individuals to be feasible. Although autologous cells may currently be used without a prospective clinical trial in the United States, there have been requirements for tracking of individual outcomes of those treated to monitor safety and efficacy. Facilities and procedures are subject to inspection by the U.S. Food and Drug Administration. There is only one large commercial group in the United States currently providing relatively large-scale processing of cartilage cells and epithelial skin grafts in the United States, although smaller enterprises at academic and small company labs are also active.

Certainly, to be accepted in the medical system, efficacy must be shown with any therapy. Therefore the up-front hurdles seem less, and certainly the immunological hurdles, as well as some technical hurdles, can be

circumvented by the use of autologous cells. But to be able to reach many thousands, robust, regulated procedures must be in place, and efficacy must be documented.

The use of allogeneic cells presents the hurdle of regulation as either a device, biologic, or in some circumstances, a drug. Once these hurdles are overcome, there are advantages to the use of allogeneic cells. The use of allogeneic cells should not be feared because of the long time lines for development and regulatory approval, because, if successful, they offer hope for an "off the shelf" or on-demand tissue of consistent quality. The use of allogeneic cells, whether derived from the adult, neonate, or developed from stem cells (non-embryonic or embryonic), will make tissue engineering or cell therapies accessible to the greatest number of individuals.

The advantages of an allogeneic cell source are availability, the ability to better control donor tissue with respect to amount and quality, the potential to develop large cell banks as a consistent starting material, the potential to propagate and otherwise manipulate the cells as needed without limiting time constraints, and the ability to select cell populations for desirable characteristics. The disadvantages are that one must deal with the potential for transmission of disease from the host cells and the immunology of allogeneic cells.

The need for extensive safety testing, in the form of donor screening and cell bank analysis, requires that methods for propagation be robust enough to yield large numbers of cells in generation of master and working cell banks. Safety testing of cell banks can exceed transplant standards and what can practically be performed on autologous cells, giving us the ability to karyotype, perform sensitive DNA analyses for infective agents at multiple points, and perform lengthy tumorgenicity testing, etc. The high costs of such scrutiny only make it feasible if multiple patients can now be treated with material derived from a single source.

As Dr. Hardin-Young discusses, the immunology of allogeneic cells is just now becoming understood. In the living skin construct, the allogeneic cells (keratinocytes and fibroblasts) do not elicit an immune response in patients. This is likely due to their inability to co-stimulate T lymphocytes—necessary for mounting a cell-mediated immune response. This finding suggests that other parenchymal cells of the body, i.e., other non-professional antigen presenting cells, could be candidates for use in tissue engineering. This concept has positive implications for the use of multi-potent cells derived from allogeneic donors as well. To date, principal groups producing autologous cell products and engineered allogeneic tissues for human use are U.S. companies.

There are times when the cell source *will* pose an immunological hurdle. This is the case with the use of xenogeneic cells. Certainly, the number of available human donors limits the use of human cells and organs. In cases where methods of cell propagation are still inadequate, xenogeneic cells have the potential to serve as a plentiful source of tissue and possibly whole organs. Dr. Cooper discusses the possible use of porcine cells and the associated immunological hurdles. The induction of tolerance by infusion of bone marrow cells is a strategy for both xenogeneic as well as allogeneic tissues and has shown some promise in primates, although there is still much to be learned.

The rapid progress in the understanding of stem cell potential, both multi-potent and pluri-potent, give human cells the potential to supersede the need for xenogeneic sources if cell propagation and differentiation techniques proceed rapidly in the next several years. The United States has commercial enterprises in xenogeneic tissue and organ technologies and therapies, as well as stem cell research in the private and public sector.

Other immunosuppresion approaches are on the horizon as well. New methods are being developed to block the important CD40 co-stimulatory signal. Clinical experience as discussed by Hardin-Young, using cells which do not naturally have a functioning co-stimulatory pathway, suggest that blockade of the pathway in cells which do, should result in acceptance by the body. This is currently the subject of both basic and clinical research in the United States.

We can see that harnessing the potential of human stem cells could significantly impact tissue engineering by providing a cell source where there often isn't one. This is presenting new biological challenges as we attempt to achieve functional differentiation and tissues from these cells.

Embryonic stem cells, or cells from the inner cell mass of the embryo, have been known for quite some time. Their unregulated growth and persistence in the body clinically presents itself as a teratoma, where such masses often exhibit a variety of differentiated cells types and structures such as hair and teeth. Control of the potential of these pluripotent cells is the challenge that is now being addressed *in vitro* by a number of U.S. laboratories, both public and private. Concurrently, it is becoming clear that cells reside in the adult, which are multi- if not pluripotent. Dr. Marshak discusses the potential of some of these cells and demonstrates that multipotent cells derived from adult bone marrow stroma, the mesenchymal stem cells, can be directed toward cartilage, bone, or muscle differentiation. Others have preliminary evidence that the bone marrow contains cells that are not just multipotent as above, but pluripotent. Surprisingly, there is evidence that adult-derived stems cells may be capable of giving rise to tissues that span the traditional ectodermal, mesodermal, and endodermal embryonic lineage.

Our knowledge of stem cell behavior is developing rapidly, although it is currently unclear whether some of the phenotypic characteristics seen are true indicators of a fully differentiated cell or merely an adaptation with nominal expression of a particular characteristic or product. For example, despite the progress in cultivation of neural stem cells and our increased understanding of neural cell lineage, the creation of a truly functional dopaminergic neuron from a serially propagated, previously undifferentiated stem cell culture shows promise but is still elusive. Efficiency and extent of cell conversion appears to be a key hurdle. Multipotent cells, partially committed to a particular cell lineage, may have a practical advantage in this area. Our ability to efficiently repopulate an organ with cells capable of restoring lost function remains the practical goal. Tissue engineering is expected to play a key role in this area.

Our ability to propagate, control, and differentiate cell populations is a recurring theme, almost regardless of the source. Dr. Block discusses his experience with the development of defined media formulations as well as with methods for cell propagation and formation of tissue masses such as pancreatic islets. Certainly, the allogeneic skin construct used as an example by Dr. Hardin-Young is enabled by our ability to propagate both the fibroblast and keratinocyte in large quantities from a single source. In addition, methodology that permits recapitulation of keratinocyte differentiation is a key element for the formation of the skin construct. We must discover how to do this with other systems.

Many other cell types are still considered non-propagatable to a large degree. Previous thinking was that many differentiated cells of the adult human have a limited capacity to divide; however, it is well established that the liver is able to rapidly regenerate in the body. Dr. Block demonstrates that it may now be possible to cultivate the human hepatocyte from adult tissue and provide conditions for maintenance of stable differentiated function. Breakthroughs in the area of defined cell propagation and organotypic or permissive culture methods, such as specialized bioreactors that promote formation of cell masses, may be needed to maintain or stimulate a cell's phenotypic potential and maximize efficacy in many instances. Dr. Block's and other U.S. laboratories, both public and private, are developing promising data on the cultivation of hepatocytes, and pancreatic islet cells in particular.

BIOMOLECULES (HOWARD GREISLER)

For purposes of the overall organization of this conference on tissue engineering, "biomolecules" are defined as all biological materials excluding cells and excluding structural proteins when they are used as the "natural biomaterials" themselves. Biomolecules include proteins, lipids, etc., and may serve vastly diverse functions key to either the assembly of, or the structural integrity of, the tissue-engineered constructs, or to the functional parameters of that construct. This diversity necessarily precludes exhaustive discussion of all biomolecules and their potential applications. Nonetheless, general categories are defined as a result of their biological function or pertain to tissue engineering applications. These include growth factors, differentiation factors, and angiogenic factors, all essential to all categories of tissue engineering, along with bone morphogenic proteins with a broad range of functional properties essential to at least hard tissue constructs. It must be recognized that the redundancy found in most biologic structures is such that precise characterization of a biomolecule as falling in just one category above is misleading. Many biomolecules may provide a host of functions and may modulate cell attachment, cell growth (or apoptosis), cell differentiation, cell migration neovascularization, etc., and indeed may do so differently according to the biochemical, cellular, and biomechanical context into which they are placed. It must further be recognized

that tissue engineering technologies may utilize either a protein itself or the gene from which that protein may be generated, in the form of a transgene product. Thus, this section of biomolecules includes a segment devoted specifically to gene delivery as a means of generating the biomolecule, which itself may be a growth factor, an angiogenic factor, etc.

While great advances have been made in technical challenges of gene transfer, these approaches continue to face significant hurdles in relation to efficiency of and cell specificity of gene transfer, regulation of expression of the transgene, and potential toxicity of either the vector or of the unregulated transgenic product itself. As Dr. Bonadio pointed out, both plasmid DNA vectors and currently unavailable viral vectors have limitations. Plasmid vectors are relatively safe but vulnerable to nuclease attack and consequent inefficiency and expense. Progress is being made by altering the surface properties of plasmids with PEG to prolong half-life and by use of targeting ligands to enhance gene transfer efficiency by receptor-mediated rather than non-specific endocytosis. Other potential strategies might include regulation of intracellular trafficking to protect the plasmid from lysosomal degradation and plasmid encapsulation in polymer scaffolds (e.g. PLGA) to protect the plasmid from extracellular nucleases.

Viral vectors including adenoviruses, retroviruses, lenteviruses, etc., increase gene transfer efficiency but themselves have limitations including possible toxicologic and immunologic responses. Recombinant adeno-associated viruses (rAAV) show some promise in transducing both dividing and nondividing cells and in persistence as integrated tandem repeats in chromosomal DNA. They can be engineered to exclude the immune response to the adenovirus and reportedly have been expressed constitutively for years in skeletal muscle and brain in animal models.

For tissue engineering approaches, porous biomaterial scaffolds including either recombinant human collagens or synthetic polylactic polyglycolic acid substrates may promote gene transfer efficiency by providing the surface for cell-DNA vector interaction. Such a gene-activated matrix (GAM) can be formulated with multiple genes with cell targeting sequences to guide the behavior of specific cell populations. Technologies exist to regulate the kinetics of bioavailability to each gene from the scaffold composites.

Toxicity issues include the interaction of the gene delivery system with the local tissue bed, vector persistence, immune responses, and the pharmacokinetics and pharmacodynamics of the vector-encoded protein, both locally and systemically. Recent work has focused on differentiation factors modulating cellular behavior. Such factors are central to concepts of stem cell biology and in adult tissues may be involved in regulating differentiation of immature pluripotent cells or transdifferentiation of other nonterminally differentiated cells. For example, evidence supports the possibility of isolating circulating primitive endothelial cells, which could then be expanded for use in tissue engineering applications. Dr. Maciag discussed regulation of endothelial cell differentiation in culture systems in which cells can be induced to form three-dimensional capillary networks and conversely de-differentiated back into two-dimensional monolayers. Considerable effort has focused on isolation of immediate early genes as mediators of cellular differentiation pathways, and more recent work in Dr. Maciag's lab has looked specifically at intermediate and late gene expression using differential display of endothelial cells in both tube and monolayer conformations. These studies have shown the importance of jaggeds, which are ligands for notch receptors intimately involved in EC differentiation processes.

Growth factors are represented by a number of families of related polypeptides with strong mitogenic activity often combined with chemotactic and differentiation properties. Among these are the FGF and the VEGF families. The FGF family is the more mitogenically potent and now numbers 22 different genes, some members having secretory signal sequences. Dr. Maciag discussed signal transduction pathway and the importance of residence times in which growth factor and cells interact. Extracellular FGF must be present throughout the G_1 transition period. Dr. Maciag further addressed the issues of FGF release into the extracellular space in the absence of a signal sequence, this release promoted by cellular stresses including heat shock, hypoxia, and possibly shear stress and strain.

For tissue engineering purposes, both growth factors and differentiation factors are likely essential to establishing a sufficient number and architecture of appropriately functioning cells. These factors may be

exogenously added, or the cells themselves may be induced to synthesize them in response to chemical and/or physical parameters.

Cellular tissue-engineered constructs (perhaps excluding those less than 100-200 μ thick, which may be oxygenated by diffusion) require a capillary network for cell maintenance and function. Our understanding of the principles of angiogenesis has recently been enhanced by investigations in developmental biology and in settings of naturally occurring angiogenesis such as wound healing, the menstrual cycle, diabetic retinopathy, and cancer. Recent efforts have addressed strategies for delivering angiogenic factors, including VEGF, FGF and others, to ischemic tissue beds to promote *in vivo* angiogenesis. These strategies may be applicable to *in vitro* engineered tissue constructs as well. Dr. Simons addressed the distinct biologic processes of angiogenesis, arteriogenesis, and vasculogenesis, each regulated by distinct mechanisms. Angiogenesis refers to newly formed capillaries likely derived *in vivo* from post-capillary venules by endothelial cell proliferation and achievable *in vitro* in endothelial cell cultures in fibrin and collagen gels. Both angiogenic factors and their receptors may be modulated by ischemia, hypoxia, and inflammation. Arteriogenesis involves formation of fully formed arteries containing all three-wall layers, a process possibly modulated in part by inflammatory mediators and shear stress. Vasculogenesis refers to development of new vasculature from pluripotent stem cells as seen in embryogenesis. Vasculogenesis may occur in adult tissues in select circumstances. Angiogenic factors may be loosely defined as a group of proteins capable of stimulating growth, migration, and/or maturation of blood vessel wall cells and generally bind to cell surface heparans. These protein families include FGF, VEGF, PDGF, TNF, angiopoietins, chemokines (IL-8, MCP-1, etc) and others; most have a diversity of other biological functions as well.

Strategies for delivery of angiogenic factors include delivery of the gene or of the protein, or use of cells themselves genetically modified to include the desired angiogenic gene. Dr. Simons pointed out the theoretical desirability of delivery of multiple angiogenic factors because of both their potential synergism and their varied mechanisms of action, but he affirmed that the current patent situation makes such an approach problematic.

Bone morphogenic protein (BMP) refers to a family of osteoinductive proteins expressed during embryogenesis and during bone fracture healing and remodeling. An important observation has been the co-dependence of fracture healing on both osteoinduction and on angiogenesis. Dr. Morris discussed the recent data on recombinant BMP-2, highly conserved through evolution. rBMP-2 acts primarily as a differentiation factor. In animal and clinical trials it has been delivered via collagen scaffolds implanted at the fracture site at the time of orthopedic or spine surgery or dental/craniofacial surgery. Dramatic osteoinduction resulted in acceleration of callus formation and maturation and decrease in time to achieving normal bone strength. For tissue engineering purposes, the BMP family members may be delivered via degradable scaffolds with cultured osteoblasts, theoretically in combination with endothelial cells and angiogenic stimuli.

References

Boden, S.D., H.S. Zdeblick, J.S. Sandhu, and S.E. Hein. 2000. *Spine* 25:376-381, 2000.

Bonadio, J. 2000, *J. Mol. Med*. In press.

Bonadio J., S.A. Goldstein, and R.J. Levy. 1998. *Advanced Drug Delivery Reviews* 33:53.

Boyne, P.J., R.E. Marx, M. Nevins, M.G. Triplett, G.E. Lazaro, L.C. Lilly, M. Alder, and P. Nummikoski. 1997. *Int. J. Periodontics Restorative Dent*. 17:11-25.

Carmeliat, P., 2000. *Nat. Med*.

Friedmann, J. 2000. *Science* 287:2163.

Gray, J.L., S.S. Kang, G.C. Zenni, D.U. Kim, P.I. Kim, W.H. Burgess, W. Drohan J.A. Windkes, C.C. Haudenschild, and H.P. Greisler. 1994. *J. Surg. Research* 57:596-612.

Jones, M.K., et.al., 1999. *Nat. Med,*. 5:1418.

Kay, M.A. , C.S. Manno, M.V. Ragni, P.J. Larson, L.B. Couto, A. McClelland, B. Glader, A.J. Chew, S.J. Tai, R.W. Herzog, V. Arruda, F. Johnson, C. Scallan, E. Skarsgard, A.W. Flake, and K.A. High. 2000. *Nature Genet*. 23: 257.

Li, J., et al. 2000. *Nat Med.* 6:49-55.

Sarikaya, M. 1999. *Proc. Natl. Acad. Sci. U.S.A.* 96: 14183.

Schaffer, D.V., and D.A. Lauffenburger. 2000. *Curr. Opin. Mol. Therapeutics.* In press.

Shea, L.D., E. Smiley, J. Bonadio, and D.J. Mooney. 1999. *Nature Biotech.* 17:551.

Xue, L., A. Tassiopoulos, S. Woloson, C. Sullivan, B. Hampton., W. Burgess, and H.P. Greisler. *J. Vasc Surg*. In press.

Xue, L., P. Shireman, B. Hampton, W. Burgess, and H.P. Greisler. *J. Surgical Research*. In press.

CELL-BASED SENSORS, OTHER NON-MEDICAL APPLICATIONS (MILAN MRKSICH)

This session considered recent work in the United States to develop and apply cell- and tissue-based technologies for nonclinical purposes. These efforts are motivated by the realization that the combination of man-made systems and biological systems, which each have unique characteristics, could yield engineered devices with broad new capabilities. A central challenge and focus for this vision is the development of a common framework for designing and building structures having both materials and biological components. This framework should make the connection between engineered systems—which are based on firm physics and engineering, use inorganic and metallic materials, and are constructed with photolithography and microfabrication tools—and biological systems—where the design rules are in many cases incompletely understood, which use soft materials in aqueous environments, and which rely in large part on self-assembly for their construction. There are two compelling reasons for including this topic in the tissue engineering study. First, the realization of cell- and tissue-based technologies will require expertise developed in tissue engineering to join cells and tissues with man-made materials and to stabilize these structures. Second, programs that integrate cell/tissue function with materials processes are certain to provide new, but longer-term, strategies for future tissue engineering applications, and in particular, for integrating prosthetic devices with tissue.

Research activity in this field is still at an early stage and lacks a concerted focus. The fundamental goals are to identify new ways of integrating cells or tissue with materials, especially in ways that provide for the fusion of biological and materials processes, and to develop standardized fabrication protocols to build these structures. The applied goals are to identify applications that could strongly benefit from devices that utilize cells or tissues as functional components. The best developed applications have used cells and tissues as the sensing elements in sensors for detection of chemical and biological agents. A second application that has attracted much interest is the construction and use of neuronal networks, but this area has not yet progressed to an application. There has been little activity to investigate other concepts that utilize the capabilities provided by cells and tissue.

Sensors of chemical and biological agents, including viral and bacterial pathogens, are important to clinical diagnostics, food monitoring, and detection of bio-warfare agents in urban and military settings. Yet, current sensors still lack the combination of selectivity, sensitivity, and response time needed for many applications, and they fall far short for real-time sensing with hand-held devices. Cells and tissues have several characteristics that make them better suited for sensing these targets. Cells present multiple receptors (some of which have low specificity for single targets) and rely on complex nonlinear information processing that allows them to identify agents with high accuracy. Cells also employ amplification schemes to improve sensitivity and reduce response times. The use of cells as sensor elements still requires that the cells be joined with a materials device and that the natural transduction mechanisms of living cells be translated to give electrical outputs from the device.

One class of approaches uses microelectrode arrays to monitor ion channel activity in adherent neuronal cells. This strategy is well suited for detecting neurotoxins and other chemical agents that act against membrane channel receptors. Several research groups have developed and fabricated integrated arrays that are tailored to these applications and have developed microfluidic cassettes that permit automated sample introduction and assays. There have also been important advances in developing pattern recognition systems that can identify with better accuracy the source of changes in electrical activity. The United States is clearly the leader in developing integrated, cell-based devices that combine sophisticated electrical and microfluidic engineering. These efforts have not, however, yet made use of sophisticated cell and molecular biology to engineer cells that respond to a broader class of agents and do so with greater specificities.

A second approach has used cells that are engineered to give spectroscopic signals in response to specific signal transduction pathways. Most strategies use cells that are transfected with green fluorescent protein (GFP) and can take many forms. Cells that are engineered to express the GFP under the control of specific promoters report on the promoter activity. In other strategies, cells are engineered such that GFP fusion proteins undergo translocation within the cell, for example, localization of transcription factors from the cytosol to the nucleus. Other strategies rely on fluorescence energy transfer between pairs of chromophores. This class of cell-based sensors offers wide flexibility in engineering cells to respond to a range of targets because they give direct information on key molecular processes within the cell. There have also been important advances in developing software architectures for storing and mining fluorescence data in order to give robust identification of targets.

Other efforts in the United States are directed towards the development of neural networks for studying fundamental aspects of learning and memory and perhaps for certain types of computation. Current efforts are developing methods to pattern neuronal cells into defined geometries on a substrate and to stimulate and record electrical activities from populations of neurons that are joined with functional synapses. Efforts are now moving towards characterizing the electrical properties of simple, but defined, neural networks.

There is substantial opportunity to investigate other modes for integrating cells and tissue with materials and for harnessing other characteristics of cells/tissues for the performance of engineered devices. One example is the potential to use mechanical activities of cardiac cells or of muscle tissue to serve as actuators in bio-microelectromechanical systems. These and many other possibilities have not yet been explored but offer exciting opportunities for both fundamental studies and engineering goals in this emerging field.

The funding for these projects has been provided by targeted programs within federal agencies. The Defense Advanced Research Projects Agency has supported programs to develop cell- and tissue-based biosensors, and the National Science Foundation has introduced special initiatives in the Engineering Directorate (XYZ on a Chip) to explore novel combinations of engineering and biological technologies. It is important to recognize that these programs were not established to provide long-term and consistent support to these research areas. There currently are no programs that are targeting the broader development of cell- and tissue-based technologies, and that are providing for fundamental research that will enable future applications. This lack of federal support remains a significant obstacle to the development of this exciting and important field.

ENGINEERING DESIGN ASPECTS (DAVID MOONEY)

Introduction

A number of engineering issues are clearly critical to the successful development of tissue-engineered products and a tissue engineering industry. These issues include elements of mass transport, biomechanics, biomaterials, and bioelectronics. The focus here is the mass transport and biomechanics design aspects, as the biomaterials and bioelectronics issues are covered in other sections.

The specific mass transport aspects deemed critical for tissue engineering include

1. Adaptation of existing bioreactor technology for large-scale cell expansion
2. Assuring sufficient oxygen and other nutrient availability to transplanted cells and those in bioreactors
3. Development of delivery vehicles for growth factors and other macromolecules to induce blood vessel formation
4. Identification of appropriate techniques for preserving both cells and engineered tissues

Relevant biomechanics issues include

1. Evaluating the critical mechanical properties of the tissues one wishes to replace
2. Determination of the minimum values of these properties required for an engineered tissue
3. Identifying the role of externally applied mechanical stimuli in the development and function of engineered tissues.

The following sections contain a very brief overview of a few of these issues. The accompanying articles in this section typically provide more extensive background to these issues and a more in depth-discussion of the current state of the art.

Mass Transport Issues

Bioreactor technology

Bioreactors are utilized in tissue engineering as a tool to generate cells for subsequent transplantation, to grow three-dimensional tissues prior to transplantation, and directly as organ support devices (see article by W. Miller in the full workshop proceedings report: http://itri.loyola.edu/te/usws/welcome.htm). Many tissue engineering strategies rely on multiplying cells from a small biopsy or starting tissue sourc and subsequently harvesting these cells for transplantation directly or on a polymeric scaffold. Earlier bioreactor technologies, which focused on growing single cells or small cell clusters, provide a suitable basis for this type of cell expansion work, which is done by a number of tissue engineering companies, including Advanced Tissue Sciences (La Jolla, CA), Organogenesis (Canton, MA), and Reprogenesis (Cambridge, MA). In certain situations, however, simultaneous culture of multiple cell types may be required, and this requires more complex bioreactor design (Emerson et al. 1991). In addition, the cultivation of three-dimensional tissue constructs places great demands on the capability of the bioreactor system to provide sufficient nutrient transport, and this is the basis for significant research (Obradovic et al. 1999). The use of bioreactors as support devices for liver or kidney function provides another layer of complexity, as transport between the cells in the device and fluids flowing through or in partial contact with these cells must be optimized (McLaughlin et al. 1999; Nikolovski et al. 1999).

Oxygen transport

It is critical that transplanted cells or cells in bioreactors have sufficient nutrient and waste exchange with their surroundings in order to survive, function appropriately, and become integrated with host tissue following implantation. Oxygen transport is typically considered the limiting factor for nutrient exchange (see article by C. Colton in the full workshop proceedings report). Tissues in the body overcome issues of mass transport by containing closely spaced capillaries that provide conduits for convective transport of nutrients and waste products to and from the tissues. It is similarly considered critical for any engineered tissue of significant size to become vascularized. Oxygen transport is a critical feature of bioreactor design as well.

There are three approaches currently being investigated to promote vascularization of engineered tissues. First, scaffolds utilized for cell transplantation are designed to promote invasion of host fibrovascular tissue by the inclusion of large, interconnected pores (Mikos et al. 1993). However, fibrovascular ingrowth into the scaffolds occurs at a rate less than 1 mm/day and typically takes one to two weeks to completely penetrate even relatively thin (e.g., 3 mm thick) scaffolds. The second, more active approach to promote vascularization of engineered tissues is the delivery of angiogenic growth factors (e.g., VEGF, bFGF) to the implant site. It has recently been demonstrated that these factors may be directly included within the tissue engineering scaffolds for a sustained delivery at the desired site (Sheridan et al. 2000). It may also be possible to utilize local gene therapy to promote vascularization by release of plasmid DNA encoding the growth factors from the tissue engineering scaffold (Shea et al. 1999). A third approach to enhance angiogenesis in engineered tissues is to co-transplant endothelial cells along with the primary cell type of interest. The endothelial cells seeded into a tissue engineering scaffold form capillaries that can merge with capillaries growing into the scaffold from the host tissue (Nor et al. 1999).

Cryopreservation

Cells, macromolecular biologically active drugs, and three-dimensional tissues grown in bioreactors will all likely be important tissue engineering products. In all three cases, it will be critical to develop technologies for the long-term, stable storage of these products following production and prior to clinical utilization. Storage typically involves reducing or removing water (e.g., lyophilization of protein solutions). The controlled transport of water from the proteins, cells, and tissues is a complex mass transfer problem. Long-term storage of protein products is an important issue in the biotechnology and pharmaceutical industries;

this has received extensive attention by these industries. Cryopreservation of cells and tissues, however, is still an emerging field with many challenges (see article by M. Toner in the full workshop proceedings report).

Biomechanics Aspects

Many of the tissues for which one may desire to engineer a replacement tissue have (a) mechanical function(s) (e.g., blood vessels, bone, cartilage). However, at the current time the mechanical properties of many of these tissues have not been precisely defined. In addition, it is unclear which of the properties, and to what magnitude, are important to use as design parameters for the engineered replacement tissues (see articles by Guilak and Nerem in the full workshop proceedings report for full discussion of this issue).

Externally applied mechanical signals are clearly regulators in the development and function of a variety of tissues. Increasing evidence from basic biology studies indicate cells mediate the response of mechanical signals. However, the increasing amount of basic information available from these studies is just now being utilized in the design of engineered tissues. The mechanical properties of many engineered tissues are inferior to the native tissue, and it has been widely hypothesized that appropriate mechanical stimulation of engineered tissues may contribute to a more natural structure and mechanical properties. Recent studies with engineered cartilage (Carver and Heath 1999) and blood vessels (Niklason et al. 1999) support this hypothesis, as mechanically stronger tissues could be formed with appropriate mechanical input.

Summary

A large number of engineering design aspects must be considered to engineer fully functional tissues. There has been considerable work recently in many of these areas, with promising results. However, significant work remains in each of these areas. It may be particularly important in the future to consider how these variables may interact with each other to control the function of engineered tissues. For example, the biomaterials and biomechanics design issues may need to be considered together in certain situations. It has recently been demonstrated that engineered smooth muscle tissues only respond to mechanical stimuli and form stronger tissues when adherent to specific types of adhesion molecules on the scaffolds (Kim et al. 1999). In addition, the mass transfer issues may have significant impact on the mechanical properties of engineered tissues, as recently described for cartilage grown *in vitro* (Vunjak-Novakovic et al. 1999).

References

Carver, S.E., and C.A. Heath. 1999. Increasing extracellular matrix production in regenerating cartilage with intermittent physiological pressure. *Biotechnology & Bioengineering* 62 (2):166-74.

Emerson, S.G., B.O. Palsson, and M.F. Clarke. 1991. The construction of high efficiency human bone marrow tissue ex vivo. *Journal of Cellular Biochemistry* 45 (3):268-72.

Kim, B.S., J. Nikolovski, J. Bonadio, and D.J. Mooney. 1999. Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nature Biotechnology* 17 (10):979-983.

McLaughlin, B.E, C.M. Tosone, L.M. Custer, and C. Mullon. 1999. Overview of extracorporeal liver support systems and clinical results. *Annals of the New York Academy of Sciences* 875:310-25.

Mikos, A.G., G. Sarakinos, M. Lyman, D.E. Ingber, J. Vacanti, and R. Langer. 1993. Prevascularization of porous biodegradable polymers. *Biotech. Bioeng*. 42:716-723.

Niklason, L.E., J. Gao, W.M. Abbot, K.K. Hirschi, S. Houser, R. Marini, and R. Langer. 1999. Functional arteries grown *in vitro*. *Science* 284:489-493.

Nikolovski, J., E. Gulari, and H.D Humes. 1999. Design engineering of a bioartificial renal tubule cell therapy device. *Cell Transplantation* 8 (4):351-64.

Nor, J.E., J. Christensen, D.J. Mooney, and P.J. Polverini. 1999. VEGF enhances the survival of endothelial cells and sustains angiogenesis by inducing expression of Bcl-2. *Am. J. Pathol.* 154:375-384.

Obradovic, B., R.L. Carrier, G. Vunjak-Novakovic, and L.E. Freed. 1999. Gas exchange is essential for bioreactor cultivation of tissue engineered cartilage. *Biotechnology & Bioengineering* 63 (2):197-205.

Shea, L.D., E. Smiley, J. Bonadio, and D.J. Mooney. 1999. DNA delivery from polymer matrices for tissue engineering. *Nature Biotechnology* 17 (6):551-554.

Sheridan, M.H., L.D. Shea, M.C. Peters, and D.J. Mooney. 2000. Bioadsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. *Journal of Controlled Release* 64 (1-3):91-102.

Vunjak-Novakovic, G., I. Martin, B. Obradovic, S. Treppo, A.J. Grodzinsky, R. Langer, and L.E. Freed. 1999. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *Journal of Orthopaedic Research* 17 (1):130-8.

INFORMATICS (PETER C. JOHNSON)

Introduction

The informatics backbone of the United States effort in tissue engineering is an essential component of our success. Its elements include both bioinformatics related to tissue engineering and the communication/ collaborative infrastructure needed to magnify the efforts of our investigators. Its importance was sufficient to make it one of the major thrust areas of the WTEC study. The portion of the WTEC workshop devoted to informatics included several critical areas, as reviewed below.

Multidimensional Assessment and Management of Tissue Information

Peter C. Johnson, MD
President and CEO
TissueInformatics, Inc.

Raymond Vennare,
Senior VP, Technology
TissueInformatics, Inc.

The process of engineering tissues ultimately requires significant knowledge regarding the tissues being replicated. Preferably, this knowledge would comprise the structural and functional elements of the tissue and reflect an organization of this information into its most readily usable form. "Tissue Information" is a concept that encompasses all of the describable components of tissue, with an emphasis on the capture of that data in digital, readily transferable form.

The tools by which tissues are mined for their information content include imaging (of all types); analysis for the location of visible structural elements; probe-dependent imaging to identify the locations of expressed genes, proteins, and metabolic regulators; and both macro and micro mechanical analyses. It is ideal when as much or all of this information can be gleaned from the same tissue specimen before it enters a research database. If built in this way, such a database exhibits the minimum of intersample variability, and true conclusions can be drawn regarding the co-dependence and causality of different tissue elements in the generation of normal and diseased states.

In order to develop robust databases of tissue information, systems for tissue acquisition must be developed that reflect the appropriate legal and regulatory concerns. Systems of tissue analysis must then measure and record tissue architecture, DNA, RNA and protein content and information regarding the origin of the tissue, such as age, race and disease state of the donor. The compilation of this data into a useful collection requires significant database and storage system design skill, as does the development of interfaces to the data to be used by researchers. Once such a system is in place, however, the information is at hand to rationally design and develop engineered tissues. In addition, the same approach can be used to provide quality assurance during the manufacture of engineered tissues.

Resolution Sciences, Inc.

Russell Kerschmann, PhD (In absentia; presented by Peter Johnson, MD)

Resolution Sciences has developed a novel method of highly registered, three-dimensional imaging of tissues (and any other material, including biomaterials). A digital file output is created that enables researchers to analyze the structure of tissue in three dimensions, to virtually cut the tissue at any angle, and to rotate the tissue in space for multiaxial viewing. The technique involves proprietary imbedding of tissue in an opaque medium after treatment with fluorescent dyes, followed by fluorescent imaging of the block surface in sequential fashion. The process of imaging before cutting the section from the block creates a highly registered three-dimensional digital data package that will be especially useful for the analysis of multisectional structures. This approach can also be used for the three-dimensional planning of engineered tissue design.

Spectral Imaging

Jeremy Lerner, PhD
President
LightForms

Spectral imaging has recently come onto the biological stage after many years of use in the defense industry and astrophysics. Its critical difference from bright field microscopy for the acquisition of digital image information is that it can detect emissions in the UV and IR ranges in addition to the visible range. In addition, while human beings process visible range light as if it were composed of weighted amounts of red, green and blue light, spectral imaging provides a continuous digital “fingerprint” that provides intensity data for each pixel of an image at each wavelength of light tested—including IR and UV. This allows more information to be captured from an image in a very discrete and queryable form, and this has been shown to enable detection of differences between cancerous and normal tissue in some instances. Spectral imaging is likely to play significant roles in image acquisition in tissue engineering at the quality assurance stage and by answering questions regarding the fate of bioabsorbable biomaterials.

Image Analysis

Anna Tsao, PhD
President
AlgoTek, Inc.

As more and more data become available from both tissues themselves and from related measures of tissue function, higher level mathematical analyses will be needed to reduce the complexity of the data and to enable multiple scales of spatial magnitude to be crossed. This problem is generally approached using higher statistical methods that enable the description of complex data using algorithms that capture the majority of variability of the data and give it predictive value. Several approaches to image analysis utilize these techniques and many have been well developed for targeting in the defense industry. These methods hold promise in the identification of subtle commonalities and differences between tissues and will play a role in engineered tissue design and quality assurance testing.

Micro-Array Systems

George Maracas, PhD
Motorola

While many aspects of tissue architecture (and even function) can be assessed using imaging and analysis techniques alone, the very critical measurement of DNA, RNA, and protein activities within cells and tissues requires different tools. One promising emerging tool is the microarray (also known as a biochip), a slide-sized array of many discrete sites to which only one DNA segment, RNA segment, or protein can bind. Up to 10,000 such sites can presently be built onto a single slide, enabling a massive, immediate analysis of the presence/activity of molecules within cells or tissues. This data can be used for the drug discovery process or

can be combined with imaging and analysis data, as indicated above, to elucidate the mechanism of development of tissue architecture—an important step for the tissue engineering industry. These methods are also likely to play a role in tissue-engineered product quality control in the future.

In Silico Biology

Donna Rounds, PhD
Physiome, Inc.

In the end, as more and more data regarding tissue architecture and cellular function within tissues are accumulated, models can be developed that will become ever more robust predictors of tissue performance. Such models can be built at the cellular level—predicting, for example, the effect of a drug on a cardiac cell ion channel—or at the tissue, organ, and systemic level. In the latter instance, whole organ function can be modeled through the rational recreation of tissue architecture into which is embedded the virtual cells and their algorithms needed to emulate the whole organ functional process. This approach will have great utility in tissue engineering in the future since it will not only support the three-dimensional architectural design of engineered tissues but will also enable *in silico* experimentation to be done to assess growth rates, vascularization rates, and the modeling of bioresorbable biomaterial behavior within tissues.

INTRODUCTION TO LEGAL AND REGULATORY ISSUES (DAVID SMITH)

Introduction

Emerging biomedical products utilizing living tissues present a new order of magnitude of complexity in their interactions with human patients. As such, they challenge established processes for protecting patients and the public health from deleterious adventitious agents, while testing the capacity of those processes to ensure timely access to beneficial therapies. At the same time, access to human tissues for purposes of medical product development—or, less benignly, for cloning or optimization of selected functional capabilities—present potentially very troubling legal and ethical issues.

In its consideration of legal and regulatory issues affecting the introduction of engineered tissue products, the WTEC tissue engineering study seeks to compare the present approaches of the relevant regulatory authorities in the United States, Europe, and Japan, together with certain national rules that may limit access to or the use of human tissue for medical applications.

This analysis has been inaugurated with an examination of the legal and regulatory status of engineered tissue products in the United States. Presentations given at this workshop addressed patenting of tissue engineering, application of the evolving approach taken by the U.S. Food and Drug Administration (FDA) to the classification and pre-market review of engineered tissue products, and emerging concerns over the use of human tissues and protection of human subjects.

Intellectual Property[9]

U.S. Patent and Trademark Office (PTO) has not established any particular criteria for patent applications claiming new methods of manipulating human tissues to produce medical therapies. However, the PTO's latest guideline outlining the inventor's obligation to demonstrate a present ability to perform the invention to achieve a useful purpose (i.e., the requirement of "enablement") may threaten the present patentability of tissue engineering methods which may be integral to new tissue therapies but which are not developed to the point of delivering those therapies.

[9] General information regarding U. S. Patent & Trademark Office policies and procedures (including the enablement and utility guidelines) as well as all patents issued since 1976 can be found at the PTO website (http://www.uspto.gov).

FDA Regulation[10]

Human tissues used for medical purposes have been classified by the FDA as "human tissues intended for transplantation" or as medical products, either as devices (as in the case of dura mater, human lenticules, and allograft heart valves) or as biologics (as in the case of blood, blood components, and blood products). Consequently, engineered human tissue products can be expected to be regulated by the FDA either as medical devices—through the Agency's Center for Devices and Radiological Health (CDRH)—or as biologics—through its Center for Biologics Evaluation and Research (CBER). However, the criteria and process for such classification and subsequent marketing review will be substantially influenced by new regulations presently in development.

Much of the regulatory framework for engineered tissue products has yet to be promulgated by the FDA through formal, binding rule-making procedures. Nevertheless, the FDA has issued a number of documents over the past few years, which, although not binding on the agency, do provide the public with a formal expression of current thinking regarding the future regulation of engineered tissue products (see Table D.1) Of these, by far the most important has been the Proposed Approach to Regulation of Cellular and Tissue-Based Products (the "Proposed Approach"), which was issued by the FDA on February 28, 1997.

Building upon the concepts and strategies set out in the Agency's 1993 pronouncements regarding somatic cell therapies and transplanted tissues, the Proposed Approach outlines a plan of regulatory oversight, which can include a pre-market approval requirement for such tissue products based on a matrix ranking the products, classified by certain characteristics within identified areas of regulatory concern. Engineered tissue products would be classified according to (1) the relationship between the donor and the recipient of the biological material used to produce the tissue product; (2) the degree of *ex vivo* manipulation of the cells comprising the tissue product; and (3) whether the tissue product is intended for a homologous use, for metabolic or structural purposes, or to be combined with a device, drug, or biologic.

The Proposed Approach also announced the establishment of an inter-Center Tissue Reference Group to act as ombudsman to resolve product classification disputes and ensure agency-wide consistency in the application of relevant regulatory authority over transplantable or engineered tissues used as medical therapies.

Presentations describing FDA's classification and pre-market review of Apligraf (Organogenesis) as a medical device and Carticel (Genzyme Tissue Repair) as a biologic demonstrated the agency is actively engaged in developing rational product approval pathways for engineered tissue products according to their classification for purposes of regulatory oversight. This approach was contrasted with the present uncertain status of such products within the European Union. The speakers did note, though, that potential inconsistencies or a lack of transparency in the application of regulatory authority over engineered tissue products would increase the complexity of introducing new medical technologies incorporating human tissues without materially advancing public health or safety.

Access to Human Tissues for Research

While critical to the general advance of medical research, access to human tissues for research or product development is highly sensitive to public disclosure of tissues taken or used without consent or under circumstances suggesting a commercial market in body parts. The absence of comprehensive federal or state legislation governing "research" tissues deprives the biomedical community of clear, consistent guidelines to follow in acquiring and using tissues, while simultaneously representing a legislative vacuum that may be filled with substantial adverse unintended consequences if done suddenly in response to some public outcry. Absent effective coordination, the initiatives of individual federal agencies to establish policies for research involving human tissues or subjects may impose conflicting requirements or expectations.

---

[10] General information regarding FDA policies and procedures can be found at the FDA website (http://www.fda.gov). Specific information regarding the activities of the lead regulatory Centers, CDRH and CBER, can be found at http://www.fda.gov/cdrh and http://www.fda.gov/cber, respectively.

Table D.1
Key Documents Re: FDA Regulation of Human Cellular and Tissue-Based Products*

1. Kessler, David A., et. al., *Regulation of Somatic-Cell Therapy and Gene Therapy by the Food and Drug Administration*, 329 N.E. J. of Med. 1169 (Oct. 14, 1993)
2. Notice: Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products (58 FR 53248; Oct. 14, 1993)
3. Notice of Interim Rule: Human Tissue Intended for Transplantation (58 FR 65514; Dec. 14, 1993)
4. Notice of Public Hearing: Products Comprised of Living Autologous Cells Manipulated *ex vivo* and Intended for Implantation for Structural Repair or Reconstruction (60 FR 36808; July 18, 1995)
5. Final Rule: Elimination of Establishment License Application for Specified Biotechnology and Specified Synthetic Biological Products (61 FR 24227; May 14, 1996)
6. Notice: Availability of Guidance on Applications for Products Comprised of Living Autologous Cells. . (etc.) (61 FR 26523; May 28, 1996)
7. Guidance on Applications for Products Comprised of Living Autologous Cells Manipulated *ex vivo* and Intended for Structural Repair or Reconstruction (May, 1996)
8. "Proposed Approach to Regulation of Cellular and Tissue-Based Products" (February 28, 1997)
9. Notification of proposed regulatory approach regarding cellular and tissue-based products (62 FR 9721; March 4, 1997)
10. Final Rule: Human Tissue Intended for Transplantation (62 FR 40429; July 29, 1997)
11. Notice: Availability of Guidance on Screening and Testing of Donors of Human Tissue Intended for Transplantation (62 FR 40536; July 29, 1997)
12. Guidance to Industry: Screening and Testing of Donors of Human Tissue Intended for Transplantation (July 29, 1997)
13. Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy (March, 1998)
14. Proposed Rule: Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products (63 FR 26744; May 14, 1998)
15. Proposed Rule: Suitability Determination for Donors of Human Cellular and Tissue-Based Products (64 FR 52696; September 30, 1999)

***** The Key FDA Documents listed in this table (with the exception of Document #1) can be obtained through the FDA website (http://www.fda.gov/cdrh or http://www.fda.gov/cber) or, in the case of documents appearing in the Federal Register, through the Government Printing Office website (http://www.gpo.gov)

Establishing Standards for Engineered Tissue Products[11]

In December 1997, with considerable FDA participation and support, the American Society for Testing and Materials (ASTM) launched a comprehensive strategy to develop standards for the production of tissue engineered medical products. Through a series of semi-annual meetings since then, the ASTM tissue engineering standards effort has provided an ongoing forum for identifying and, through a careful consensus-building process, addressing the critical details essential to a thorough characterization of engineered tissue products for regulatory review. These meetings draw together FDA reviewers, industry representatives, researchers and other interested persons. Many draft standards are in various stages of development.

Conclusions from the Presentations

Taken as a whole, the presentations on legal and regulatory issues revealed that

- The pace and direction of the development and clinical introduction of engineered tissue products can be affected by many federal agencies;
- General disengagement of the biomedical community from the policy making processes of these agencies can deprive them of an important perspective on proposed actions;
- As the FDA evolves its strategy for managing engineered tissue products, it should emphasize cross-Center consistency in product classification and product approval paradigms which respond to the particular attributes and challenges of products incorporating living human tissues; and
- The FDA's effort to develop a rational approach to the regulation of engineered tissue products is well-begun; it should be continued and expanded globally through international harmonization programs.

[11] General information regarding the ASTM tissue-engineered medical products standards development effort can be obtained at the ASTM website at www.astm.org (go to the page for Committee F04, Division IV).

APPENDIX E. THE FEDERAL INVESTMENT IN TISSUE ENGINEERING[1]

Federal Government support for research has positioned the United States as a leader in global science and technology advances, particularly in biomedicine and associated fields. Indeed, most departments and agencies of the Executive Branch fund projects and programs as extensions of their core missions. By virtue of its highly multidisciplinary nature, tissue engineering occupies an important and growing place within many of these institutions. The "culture" at each organization may be relatively unique, and so a harmonized definition of tissue engineering may be difficult to establish. Additionally, different groups track and/or report their annual spending differently, making cross-agency comparisons difficult for any specific year. For example, for the purposes of tracking funding trends, the Advanced Technology Program (ATP) of the National Institutes of Standards and Technology (NIST) presents commitments to annual awards as a lump sum package in the first funding year, while the National Institutes of Health (NIH) and the National Science Foundation (NSF) calculate their investment histories based on funding to each recipient by fiscal year. Nevertheless, information about the funding history at each of the agencies by year and subtechnology area can show important overall trends in the Government's strategic investment in science, engineering, and technology.

Indeed, the U.S. Federal Government was, in many ways, the "midwife" of the tissue engineering industry, for it was an NSF panel meeting in the spring of 1987 that produced the first documented use of the term. Investigations on the biocompatibility of biomaterials and growth conditions enabling the functional culture of living cells for transplantation were central to bioengineering for decades. However, the NSF panel, and several follow up meetings in late 1987 and early 1988, defined this new and emerging technology, but more importantly, set out to fund it (Heineken and Skalak 1991). Several awards were made under NSF program announcements in 1988, 1989, and 1990 (Table E.1). Support for tissue engineering activities has been relatively steady or increasing at NSF since then, via awards from the Bioengineering and Environmental Systems Division to individual investigators, as well as in projects within three of the Engineering Research Centers (ERCs). From 1988 to 2000, $24.7 million was awarded, including $5 million in 1999 and $7 million in 2000. Over the years NSF has supported the following areas: gene therapy/gene transfer, scaffolding, cell culturing, cell adhesion, DNA delivery, stem cell technology, functional tissue engineering (e.g., mechanical properties of tissues), tissue preservation, and tissue engineering conference funding.

Many projects in the NIST laboratories broadly support the infrastructural standards and measurement needs in tissue engineering. The NIST laboratory investment in tissue engineering for FY02 is over $3 million, and is expected to grow. Moreover, to enhance cross laboratory communication and coordination of activities in tissue engineering and related areas, an intra NIST working group, Measurement and Materials at the Biointerface (MMBI), was formed in 1999. The Advanced Technology Program (ATP) became NIST's extramural funding arm and began issuing awards in 1990. From 1990 through 1995, ATP issued seven tissue engineering awards (Table E.2). Tissue engineering was one of the very promising emerging technologies highlighted at a NIST workshop in January 1994, and a follow-up workshop in late 1994 entitled, "Tissue Engineering: From Basic Science to Products," produced over 50 white papers from 80 organizations (Galleti, Hellman and Nerem 1995). There were 56 submissions to ATP's 1997 focused program solicitation in tissue engineering (97-07), and twelve awards were made. While ATP's competition model currently does not include focused programs, the fundamental concept of supporting projects that coalesce into synergistic technology areas is a core value. Tissue engineering proposals have continued to fare well, with nine awards in 1999 totaling $16.9 million and nine awards in 2000 totaling $17.4 million which brings the ATP/NIST investment in tissue engineering through Fiscal Year 2000 to $79.9 million (Table E.2). ATP extramural funding by topic within tissue engineering is presented in Figure E.1. In addition, there is a small intramural program funded by ATP that adds support to this extramural funding.

---

[1] Members of the Multi-Agency Tissue Engineering Science (MATES) Working Group of the National Science and Technology Council (NSTC) contributed to this appendix. See the list of MATES members on p. 214.

Table E.1
NSF Funding for Tissue Engineering (1988 - 2000)

Year	Individual Awards	Engineering Research Centers		
	Bioengineering Division	**Georgia Tech**	**UWEB**	**MIT**
1988	$584,000			
1989	$723,000			
1990	$710,000			
1991	$826,000			
1992	$1,458,000			
1993	$1,283,000			
1994	$1,242,000			
1995	$1,216,000			
1996	$780,000			
1997	$1,124,000			
1998	$1,494,000	$965,000	$57,000	$100,000
1999	$3,431,000	$1,118,000	$166,000	$800,000
2000	$3,910,000	$1,768,000	$137,000	$800,000
total	**$18,781,000**	**$3,851,000**	**$360,000**	**$1,700,000**
NSF Grand Total	**$24,692,000**			

Table E.2
ATP/NIST Extramural Awards in Tissue Engineering

Year	Number of Awards	ATP Funds
1991	1	$ 1.2 million
1992	1	$ 2.0 million
1993	2	$ 6.3 million
1994	2	$ 4.0 million
1995	1	$ 2.0 million
1996	0	0
1997	11	$20.6 million
1998	5	$ 9.5 million
1999	9	$16.9 million
2000	9	$17.4 million
TOTAL	**41**	**$79.9 million**

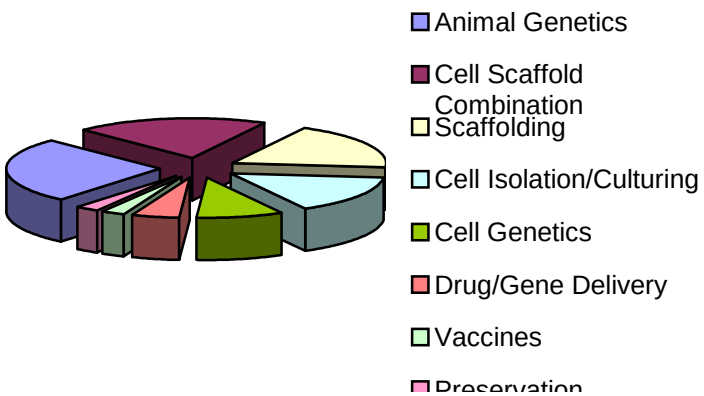


Fig. E.1. ATP extramural awards in tissue engineering by topic.

The Defense Advanced Research Projects Agency (DARPA), acting as one of the branches of the Department of Defense, funds high-risk, usually long-term research that is seen to be beneficial in military applications. The role of tissue engineering is most prominent in DARPA's Broad Agency Announcements (BAAs) addressing tissue-based biosensors, in which cells and/or tissue are the core of sensors that act as early warning detectors for chemical and biological warfare agents. DARPA's focus has been refined in this area. The most recent BAA, entitled, "Activity Detection Technologies," encompasses any type of sensor that could detect a physiological effect, whether from tissue engineering or another technology. DARPA's cumulative investments in these areas have totaled $59 million since 1998. Tissue engineering also plays a role in DARPA programs for biological warfare countermeasures and in new initiatives such as metabolic engineering for cellular stasis.

The NIH of the Department of Health and Human Services (DHHS) has been the major Federal funding agency for biomedical research for the last half century. Thus, NIH's investment in the infrastructure of tissue engineering has been long-standing and substantial. However, only recently has NIH specifically targeted tissue engineering research through grant initiatives. In 1997 NIH released its first tissue engineering initiative entitled, "Tissue Engineering, Biomimetics, and Medical Implant Science." This solicitation invited applications aimed at designing and engineering natural and novel approaches for the repair, restoration, and replacement of tissues and whole organs based on a comprehensive scientific understanding of biological structures and their function. Four major areas of research were funded, including: (1) *in vitro* engineered tissues for repair or replacement; (2) design of matrices/scaffolds for tissue replacement; (3) drug/cell/gene polymer delivery systems; and (4) fundamental cellular, molecular, and immunological research on engineered tissues.

A search of the NIH awards database (Computer Retrieval of Information on Scientific Projects—CRISP) revealed a rising profile for NIH-funded tissue engineering grants, partially due to the initiative released in 1997. In 1997 $3.7 million was awarded (in 23 grants); in 1998 there were 97 awards totaling $18.9 million; 117 projects totaling $24.6 million were awarded in 1999; and $33.6 million was awarded for 157 projects in 2000 (Table E.3). In June 2001, the NIH Bioengineering Consortium (BECON), which is the central focal point for biomedical engineering activities at NIH, held a symposium entitled, "Reparative Medicine: Growing Tissues and Organs." The recommendations for future research directions in this field that resulted from the symposium will be implemented in the near future. Research initiatives in this area will be coordinated, in part, by the new National Institute for Biomedical Imaging and Bioengineering (NIBIB) at NIH.

Table E.3
NIH Funding in Tissue Engineering

Year	Number of Grants	FY Funding
1997	23	$ 3.7 million
1998	97	$18.9 million
1999	117	$24.6 million
2000	157	$33.6 million
TOTAL	**394**	**$80.8 million**

The Food and Drug Administration (FDA) is a sister agency of NIH in DHHS. While the FDA does not issue extramural awards to study any particular field, the agency is pivotal in the nation's health care system, due to its regulatory role. Technology forecasting and monitoring for its science-based product evaluation/review and approval process is central to FDA's function. Since the research/review partnership is critical in the regulatory process, FDA supports intramural research programs in bioeffects studies and test method development directed towards safety and effectiveness considerations of tissue-engineered combination medical products. This is reflected in the activities of several intramural scientists involved in research that inform and extend the broad area of tissue engineering, and in FDA's regulatory and policy development activities. For example, FDA scientists participate in organizations that foster the exchange of scientific information to further the field and in organizations that are interested in the development of standards.

FDA currently maintains active regulatory research programs in the area of tissue engineering. FDA's intramural research effort was approximately $1.5 million in 2001 and is estimated to be $1.8 million in 2002. The research includes programs in genomics and proteomics using microarray technology, to monitor changes in gene and protein expression during manufacturing and clinical use of biological products, and to study gene expression profiles in immunotoxicology and diagnostics. Research efforts in immunology, developmental biology, stem cells, and virology are also focused on the numerous cell types and other biological products used to prepare tissue-engineered products. In addition, biomaterials-related studies such as biomaterial characterization and test method development, and biomaterial-host cellular interactions as determined by immunological and histopathological endpoints, are actively being pursued. The collective goal of the FDA intramural research program is to provide expertise in validation of regulatory tests, including development of relevant reference standards and methodologies to ensure that tissue-engineered products are safe and effective.

In addition, FDA directs many resources toward review and regulatory activities. In 2001, FDA supported 85 full-time equivalent (FTE) staff members at a level of approximately $9.9 million in regulatory and policy development for activities related to tissue engineering. These activities included premarket review, clinical evaluation, and program management. Tissue engineering-related regulatory activities for FDA are also reflected in resources devoted to implementation of the Human Tissue Program. In 2001, FDA devoted $4.353 million to this program to support activities in regulation and guidance development, inspections, compliance, registration programs, and premarket review. FDA continues to regard this program as an important contribution to public health and continues to support efforts to ensure proper oversight and development of products in this area. The total FDA intramural effort in tissue engineering for 2001 was $15.75 million.

While there is no formal program in tissue engineering at the National Aeronautics and Space Administration (NASA) or the Department of Energy (DOE), many projects in these agencies involve tissue engineering principles and practices. Examples are the multiple uses being found for the microgravity bioreactor developed at NASA.

In summary, the cumulative Federal Government investments with sustained efforts in tissue engineering and related activities through 2000 have totaled $24.7 million from NSF, $79.9 million from ATP/NIST, $80.8 million from NIH, and $59.0 million from DARPA. In addition, FDA spent $15.75 million in 2001, the result of several years of increasing FDA involvement in the field. As technologies rapidly advance in so

many areas of this new intersection of engineering and biology, the research dollars will surely increase, as they have across the Government since 1988. New developments in combinatorial methods to discover new biomaterials, cell cycle control and stem cell biology, responsiveness of cells in their extra-cellular matrix, mass culture of cells with defined phenotypes, gene expression profiles in response to cytokines, and so much more will keep scientists and those who fund them actively engaged for the foreseeable future.

References

Galletti, P.M., K.B. Hellman, and R.M. Nerem. 1995. *Tissue Engineering* 2:147.

Heineken, F.G., and R. Skalak. 1991. *J. Biomech. Eng*. 113:111.

Skalak, R., and C.F. Fox (eds). 1988. *Tissue Engineering, Proceedings for a Workshop held at Granlibakken, Lake Tahoe, California.* Alan Liss: New York.

Members of the
Multi-Agency Tissue Engineering Science (MATES) Working Group
Active During the WTEC Study

Joseph Bielitzki, DARPA

Mrunal Chapekar, NIST

Dean Cole, DOE

Steven Davison, NASA

Joyce Frey Vasconcells, FDA

Frederick Heineken, NSF

Kiki Hellman, FDA

Kurt Henry, DARPA

Angela Hight-Walker, NIST

Rosemarie Hunziker, NIST*

Christine Kelley, NIH

Rachel Levinson, OSTP

Alan Rudolph, DARPA

David Thomassen, DOE

Darin Weber, FDA

Steven Zullo, NIST

* Active member until November 2001